INVESTIGATING PHYSIOLOGICAL AND QUALITY RESPONSE OF POMEGRANATE FRUIT TO CONTROLLED ATMOSPHERE STORAGE

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DECLARATION

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ABSTRACT

Pomegranate (*Punica granatum*) is a highly valued crop with a huge economic potential. It is very nutritious with numerous bioactive compounds with the potential to prevent a certain type of cancers and other health conditions. The fruit can also be processed into various industrial products. In South Africa, the pomegranate industry is growing rapidly due to the rising demand from local and international markets. However, owing to its high perishability, the shelf life is limited to less than eight weeks under cold storage conditions. In light of this, a study of 'CA' storage as a supplementary postharvest treatment was investigated. The 'CA' storage is a system that involves altering and maintaining an atmospheric gas composition different from that of room air (79% N_2, 20% O_2, and 0.03% CO_2) to levels generally, with O_2 below 8% and CO_2 above 1% supplemented with low temperature and relative humidity above 90%.

The primary objective of this study was to investigate the physiological and quality response of pomegranate (weight loss, shrivel, decay, colour, texture, incidences of disorders and decay) to different 'CA' treatments and storage temperatures. In addition, to assess the overall quality (total soluble solids, pH, acidity, antioxidant properties, aroma volatility compounds and sensory analysis) including respiration and development of a model for predicting the transpiration rate with the view to optimise the 'CA' storage requirements for 'Wonderful' and 'Bhagwa' pomegranates.

The results showed that the selected pomegranate cultivars responded differently to 'CA' storage conditions. More importantly, it was observed that by increasing CO_2 and/or decreasing O_2 concentrations under 'CA' resulted in opposite effects on quality attributes. Despite the variation in response, the shelf life of the whole pomegranate fruit was extended by five months more after harvest with a minimal loss of physiological properties (weight loss, physiological disorders, colour, and texture) and quality. Furthermore, the notable fluctuation of quality attributes (TSS, aroma volatility compounds and antioxidants) under 'CA' conditions depended highly on cultivar, storage temperature, gas composition and storage duration. In addition, a mathematical model developed based on respiratory heat energy reliably predicted the transpiration rate, with an accuracy of R^2 = 0.97. In conclusion, the study proposes a goal-oriented optimisation strategy focussing on the market-driven quality expectation with a negligible compromise on some attributes. The concept was validated using a general linear model (statistical tool) which showed that atmosphere under CAs minimised the weight loss with minimal physiological and quality loss of attributes in addition to extending the shelf life for than two-fold compared to room air. These findings highlight both importance and challenges in finding optimal 'CA' for the storage of fresh horticultural produce.

OPSOMMING

Granate (*Punica granatum*) is ’n waardevolle vrug met baie ekonomiese potensiaal. Dit is baie voedsaam en daar is baie bioaktiewe samestellings wat die potensiaal besit om sekere tipes kanker en ander ongesteldhede te verhoed. Die vrug kan ook in sekere industriële produkte gebruik word. Die granaatindustrie in Suid-Afrika groei tans vinnig as gevolg van die groeiende vraag na granate in plaaslike en internasionale markte. Maar omdat granate baie onderhewig is aan bederf, is die raklewe daarvan beperk tot minder as agt weke as dit onder koel toestande geberg word. Daarom is berging onder beheerde atmosfeeer toestande ‘CA’ ondersoek. Die ‘CA’ -berging behels verandering endie behoud van ’n atmosferiese gas samestelling wat verskil van dié van normale lug (79% N_2, 20% O_2, and 0.03% CO_2) tot vlakke waar die O_2 onder 8% en die CO_2 bokant 1%, die temperature laag en die lugvoggehalte bo 90% is.

Die hoofdoel met hierdie studie was om die fisiologiese en gehalte respons van granate (verlies aan gewig inkrimping, aftakeling, kleur, tekstuur, en siektetoestande) tot verskillende ‘CA’ behandeling en bergingtemperature te ondersoek. Verder is daar gepoog om die algehele gehalte (oplosbare vastestowwe, pH, suurinhoud, antioksidantkenmerke geur en sensoriese ontleding) insluitende respirasie te assesseer en om ’n model vir die voorspelling van die uitwasemingkoers te bou om sodoende die ‘CA’ berging van die kultivars, ‘Wonderful’ en ‘Bhagwa te verbeter.

Die resultate het bewys dat die verskillende tipes granate verskillend op ‘CA’ berging reageer. Meer belangrik is die feit dat die verhoging van CO_2 en/of die vermindering van O_2 konsentrasies teenoorgestelde uitwerkings op die gehalte van die vrug het. Ten spyte van die verskillende reaksies is die raklewe in die geheel met vyf maande na die oes verleng en met minimale verlies aan fisiologiese kenmerke (verlies aan gewig, fisiologiese probleme, kleur en tekstuur) en gehalte. Verder is daar bevind dat die fluktuasies in gehalte (TSS, geur, vlugtigheidsamestellings en antioksidante) onder bergingstoestande tot ’n groot mate afhang van die kultivar, bergingstemperatuur, komposisie van die gas en die duur van die berging. Verder is ’n wiskundige model ontwikkel wat baseer is op respiratoriese hitte energie wat die uitwasemingskoers koers met ’n akkuraatheid van R^2 = 0.97. voorspel. Daar is tot die slotsom gekom dat daar van ’n doelgerigte optimasie strategie gebruik gemaak moet word met die fokus op markverwagtings en met toegewens in sommige onbelangrike opsigte. ’n Lineêre model (statistiese instrument) is gebruik om die konsep te valideer. Daar is bevind dat berging onder beheerde atmosfeer tieatande lei tot minimale verlies aan gewig, kenmerke en gehalte en dat dit die raklewe twee keer meer as normale lug. Hierdie bevindinge beklemtoon die belangrikheid sowel as die uitdagings na die soeke van ‘n optimale ‘CA’ vir vars hortologie produkte.

Dedication

~ for Ninziye the only 'baby girl'

You always prayed for Dad to be safe

I could not thank you more than this~

TABLE OF CONTENTS

Language and style used in this dissertation are in accordance with the requirements of the International Journal of Food Science and Technology, as prescribed by the Department of Food Science, Stellenbosch University.

This dissertation represents a compilation of chapters in the form of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Results from this dissertation proposed for publication in the following journals:

1. Controlled atmosphere storage of pomegranate: A review. (**Food and Bioprocess Technology).**

2. Response of whole ('Wonderful' and 'Bhagwa' pomegranate) to controlled atmosphere and Xtend® film packaging conditions. **(Postharvest Biology and Technology)**.

3. Impact of 'CA' and storage conditions on antioxidant properties of 'Wonderful' pomegranate. **(Scientia Horticulturae).**

4. A kinetic model of transpiration in controlled atmosphere storage of 'Wonderful' pomegranate. (**Postharvest Biology and Technology**).

5. Characterisation of aroma and flavour quality of 'CA' stored 'Wonderful' pomegranate fruit and sensory analysis. **(Food Packaging and shelf life**).

6. Optimisation of 'CA' and storage temperature requirements for selected pomegranate cultivars. (**Postharvest Biology and Technology**).

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CHAPTER 1

INTRODUCTION

Pomegranate (*Punica granatum*) a highly valued crop with a huge economic potential. It is highly nutritious with several bioactive compounds believed to prevent a certain type of cancers and other health conditions (Seeram *et al.,* 2006; Stover & Mercure, 2007; Viuda-Martos *et al.,* 2010; Mohammad & Kashani, 2012; Teixeira da Silva *et al.,* 2013; Viladomiu *et al.,* 2013).). The fruit can be consumed as fresh and/or processed into numerous products including tea, juice blends, nut mixes and countless other food and non-food stuff (Opara *et al.,* 2009; Calın-Sanchez *et al.,* 2011; Dhinesh & Ramasamy, 2016). Owing to these qualities and economic benefits, several countries are aggressively growing (LaRue, 1980). In South Africa, the pomegranate industry is growing rapidly with roughly over 1 400 ha commercial orchards (POMASA, 2015). A remarkable increase in export was recorded by volumes ± 315 tonnes in 2011 to 198 000 tonnes in 2012, and further increase by 40% in 2014 season targeting export market mainly Europe, the Far East and Canada (POMASA, 2015). The economic outlook report No.17 of 2015 projected an increase of 189% growth in the pomegranate industry by 2017.

However, pomegranate fruit is a highly perishable fruit with shelf life limited to less than eight weeks under cold storage conditions (Nanda *et al.,* 2001; Hess-Pierce & Kader, 2003; Porat *et al.,* 2009). To facilitate off season marketing and increased consumption when fruit attracts higher prices for growers, the use of CA' offers the potential to minimize loss in quality and extend shelf life of pomegranate fruit (Kupper *et.al.,* 1995; Artes *et al.,* 1996; Hess-Pierce & Kader, 2003; Defillipi *et al.,* 2006). The 'CA' storage is a system that involves altering and maintaining an atmospheric gas composition different from that of normal air (79% N_2, 20% O_2, and 0.03% CO_2) to levels generally, with O_2 below 8% and CO_2 above 1% supplemented with low temperature and relative humidity above 90% (Dilley, 2010). Although there is considerable literature on the use of 'CA' storage in South Africa for a wide range of fruit (apples and pears) (Eksteen & Truter, 1986), reliable information is lacking for its use on pomegranate cultivars. In addition, the effects of 'CA' on bioactive compounds and volatile components important to consumer have not been reported. Currently fruit marketing is limited to the harvest period due to high incidence of weight loss, shrivel and decay which have both quality and economic implications.

Therefore, the aim of this study was to investigate the physiological and quality response of pomegranate (weight loss, shrivel, decay, colour, texture, incidences of disorder and decay) to different 'CA' treatments and storage temperatures. In addition, to assess the overall quality (total soluble solids, pH, acidity, antioxidant properties, aroma volatility compounds and sensory analysis) including respiration and development of a model for predicting the transpiration rate with the view to optimise the 'CA' storage requirements for 'Wonderful' and 'Bhagwa' pomegranates.

Objectives

i. Determine physiological and quality response of 'CA' stored pomegranate fruit;
ii. Determine the impact of 'CA' and storage conditions on antioxidant properties of 'Wonderful' pomegranate;
iii. Develop a mathematical model to predict physiological responses (transpiration) of cv. 'Wonderful' pomegranate;
iv. Evaluate the impact of 'CA' storage on aroma profile and flavour of cv. 'Wonderful' pomegranate fruit and sensory analysis;
v. Optimise 'CA' and storage temperature requirements for selected pomegranate cultivars.

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CHAPTER 2

CONTROLLED ATMOSPHERE STORAGE OF POMEGRANATE: A REVIEW

Abstract

Pomegranate is a highly perishable fruit with a shelf life less than eight weeks under the cold chain storage. The major factors that limit prolonged storage include weight loss, shrinkage, incidences of mould and decay. The current storage practice using polyethylene type of packaging materials are only suitable for a short-term storage and transportation, consequently, limit pomegranate marketing to harvest periods only. Controlled atmosphere (CA) offers a viable option than any other methods including the chemical based which are facing restrictions on the international trade. The aim of this review was to evaluate current storage practices used for pomegranate and its challenges, examine empirical studies done on the use of 'CA' technology for pomegranate and the relevant impact on quality. In addition, offer an opinion on future prospects of the 'CA'' storage method. It is evident that 'CA' storage has the potential to extend the shelf life of pomegranate, although not much has been reported on its impact on some quality attributes such as antioxidant activities and aroma volatile compounds. As a result, there is a scope for research in these areas and development of models for respiration and transpiration rates, which have an impact on physiological quality. In addition, the ongoing restriction of the use of chemical based fungicides to extend the shelf life of horticultural produce merits further the need to investigate 'CA' as an alternative safer environmentally friendly technology.

Keywords: controlled atmosphere, pomegranate, physiology, quality, shelf life

Background

Controlled atmosphere (CA) refers to an agricultural storage method in which the concentrations of oxygen, carbon dioxide and nitrogen, as well as temperature and relative humidity of the room, are regulated. The origin of 'CA' dates back to several centuries ago prior to understanding the role of O_2 and CO_2 in the basic respiration of plants and plant organs (Dilley, 2010). Bernard (1821) documented the first recorded 'CA' storage. Noticeably, fruits harvested and stored in the atmosphere with no O_2 did not ripen, but once exposed for a short period in the air continued to ripen. Later, Kidd & West (1938) made a significant contribution

to the fundamental knowledge of the role of temperature, O_2, CO_2, and ethylene in controlling fruit ripening. However, much is yet unknown particularly the effect of 'CA' on the bioactive, aroma and volatile compounds. Kader (1986) underscored the need to shift postharvest technology research towards flavour quality than physiological appearances because of consumer preference. To our knowledge, there is lack of fundamental knowledge on the effect of 'CA' on volatile compounds (Caleb *et al*. 2015), and the impact on antioxidant activities of pomegranate, there is scope for research in these areas for different pomegranate cultivars. Therefore, as the refinement of 'CA' continue taking advanced latitude covering new cultivars, the relevant empirical studies should be inclusive of all vital pomegranate quality attributes. In view of the above, this review explores in detail the advances of 'CA' technology and its relationship to the growing need for its wide application. The South African pomegranate industry is growing rapidly with no reliable information on the postharvest storage method to extend the shelf life and maintain quality of pomegranate cultivars. Hence, the research and development of 'CA' storage systems will provide a sustainable all year round supply of pomegranate to local and export market.

Types of 'CA's and room conditioning

There are two types of 'CA' s systems used by industries. The 'static-type' and 'purge-type' systems. The 'static' type relies on the product generating its atmosphere through natural respiration process, whereas, the 'purge-type 'relies on the gas flow from the external source to flush excess O_2 with N_2 in the system and stabilises within 24 hours (Drake & Eisele, 1984). However, when using biological methods (fruit respiration) to a steady state it takes within 15-25 days, with a slow and progressive decrease thereof. It is evident that a non-biological system of flushing O_2 with nitrogen (N_2) lowers O_2 level faster to 6-8% within 24 h, and then later lowering to the desired levels for storage through respiration (Yahia, 2009). In some cases, the system may be designed to utilise the flushing operation initially to reduce the O_2 content rapidly, then either injecting CO_2 or allowing it to build up through respiration and then maintenance of this atmosphere by ventilation and scrubbing (Yahia, 2009). The following section provides additional details of the role of each gas in the 'CA' systems.

Oxygen (O_2)

Oxygen O_2 is a colourless and odourless gas occupying 21% of the total air. At this concentration in room air, potential acceleration of physiological disorder and/or an immediate compositional change in fruit occurs (Artes *et al.*, 1996). A number of empirical studies involving 'CA' have recommended a rapid removal of excess O_2 to reach an optional level to prevent deterioration (Allen, 1998; Bishop, 1996). For pomegranate, a range between 2-5% O_2 in 'CA' system has recommended for some cultivars, whose optimal levels vary depending on the cultivar and geographic location where the fruit are grown (Kupper *et al.*, 1995; Kader *et al.*, 2000; Kader, 2006; Defilippi *et al.*, 2006). For example, the shelf life of 'Hicaz' pomegranate was prolonged in 'CA' with 3% O_2 combined with optimal carbon dioxide for 6 months after harvest (Kupper *et al.* 1995). Other pomegranate cultivars such as 'Mollar' and 'Wonderful' performed fairly well in the 'CA' with 5% O_2, and optimal CO_2 depending on the cultivar (Artes *et al.*, 1996; Hess-Pierce & Kader, 2003; Deffilipi *et al.*, 2006). More recently, Matityahu *et al.* (2016) studied the effects of regular and controlled atmosphere storage (2% O_2 + 5% CO_2). These results provide a good example of the variation in response of pomegranate to different 'CA' gas conditions, and that, no single 'CA' is entirely suitable for all cultivars (Saltveit, 2003).

Carbon dioxide (CO_2)

Carbon dioxide (CO_2) is a colourless and odourless gas. It has been extensively noted in the literature that optimal 'CA' has potential to extend the shelf life of horticultural products, and in particular pomegranate (Kupper *et al.*, 1995, Artes *et al*., 1996, Defillipi *et al.*, 2006). The reason for the success in extending shelf life is based on the influence of 'CA' to lower respiration rate and other biochemical activities. Hess-Pierce & Kader (2003) proposed that 10-15% CO_2 as optimal for extending shelf life different pomegranate cultivars. However, the reality is that some pomegranate cultivars performed better under 'CA' with as low as 5-6% CO_2 gases in combination with optimal O_2 levels (Kupper *et al.*, 1995; Artes *et al.*, 1996). More recently, three other pomegranate cultivars responded favourably well under 'CA' with a low level of CO_2 (Matityahu *et al.* 2016) than the level recommended in the literature. Thus, repetitive empirical studies are essential for each cultivar to investigate their physiological responses on respiration and transpiration rates in order to optimise storage conditions.

Nitrogen (N_2)

Nitrogen is the most abundant component in air (79%). Its relevance in 'CA' includes acting as a filler gas to displace excess O_2 from the air, delay the oxidative rancidity and as an alternative to inhibiting the growth of aerobic microorganisms in vacuum packaged products (Kader *et al.*, 2006).

Gas regulation in 'CA' system

The regulation of oxygen and carbon dioxide levels along with the regulation of temperature is known as controlled-atmosphere storage. In modern 'CA' storerooms, gases are regulated by computers, which monitor levels using an infrared gas analyser to measure the gas content and ensure that - levels of CO_2 and O_2 are within the set values. Through this technique, any possible build-up of either CO_2 or depletion of O_2 is averted to lessen chances of anaerobic respiration to occur during storage (Deffilipi *et al*, 2006; Kader *et al.*, 2006). The tolerance limit for gases is usually set at ±1 percentage so that, should the level go beyond/below 1 % O_2 air automatically gets injected until it reaches 1.1 %. As the level of CO_2 in the 'CA' increases through respiration, active scrubbing absorbs the excess CO_2 levels (Kader *et al.*, 2006).

Scrubbers in 'CA' system

A scrubber is compounds with the potential to absorb excess gases in the 'CA' environment. By incorporating the scrubber in the 'CA' systems, the gases are stabilised within the optimal range and respiration rate is controlled (Zagory & Kader, 1988). Scrubbers are frequently used in the absence of an automated gas flushing system the simplest method is to place within the environment a CO_2 absorbing chemical such as calcium hydroxide ($Ca(OH)_2$ that can keep the level of CO_2 within the required levels during the respiration of the fruit. The mechanism of absorbing the gas follows the following two equations (1 and 2).

Equation (1) occurs during respiration of the fruit in the atmosphere, which produces CO_2, respiratory heat energy and water as by-products of respiration.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6\,CO_2 + 6H_2O + 2816\,kJ \qquad (1)$$

In the second equation, the product CO_2 reacts with the scrubber $Ca(OH)_2$ to produce a solid $Ca(CO_3)$, thus stabilising the atmosphere;

$$Ca(OH)_2) + CO_2 = CaO_3 + H_2O \quad (2)$$

Equation 2 describes the mechanism of active scrubbing system. For more stability and long-term transportation system, passive scrubbing using dry lime bags in the storeroom can perform the same functions, although the limitation is linked to the volume occupied by the scrubber (Thomson, 1990).

Biological basis of 'CA' technology

The biology of 'CA' can be examined in the context of its relationship with the transport system within the fruit and vegetables. Once detached from the main tree the fruit continues as a living organism but the process of catabolism begins immediately leading to several compositional changes due to respiration and transpiration rates (Kader *et al.,* 2006). In principle, the biological effect of 'CA' with a defined low O_2 and/or elevated CO_2 levels has a significant impact on respiration and ethylene production in fruits (Pierce-Hess & Kader, 1984). While the vast amount of research has been done on the optimal gas composition in 'CA' for different cultivars (Kupper *et al.*, 1995; Artes *et al.*, 1996; Deffillipi *et al.*, 2006), the nature of the variability in response of pomegranate cultivar remains a challenge in that each pomegranate cultivar ought to be investigated separately. The interactive effect of 'CA' and storage temperatures at different levels has been reported in the literature (Ben-Arie *et al.*, 1984; Kader *et al.*, 1984; Yahia & Kader, 2010; Thomson, 2010), and a summary presented in Table 2.1.

Table 2.1. Comparison between optimal and sub-optimal effect of 'CA' on pomegranate fruit quality

Storage condition	Quality of pomegranate	References
Optimal 'CA'	• Reduction of the severity of chilling injuries reduced scalds and maintenance of colour of pomegranate.	Zagory & Kader (1989), Kupper *et al.* (1996).
	• Retards senescence and fruit in climacteric fruits.	Artes *et al.* (1996)
	• Retards growth of gray mould caused by Botrytis cinerea, reduce decay in fruits.	Yahia (1998). Thomson (1998)
	• Retards biosynthesis and oxidation of phenolic compounds, carotenoids and anthocyanins.	Hess-Pierce & Kader (2003)
	• Slows down activities of cell wall degrading enzymes involved in softening of fruit.	Defillipi *et al.* (2006)
	• Retention of Ascorbic acid and other vitamins resulting in better nutrition	Palour *et al.* (2006)
	• Oxygen level below the optimal threshold affects flavour.	Kader (2003) Nerya *et al.* (2006)
'CA' -outside optimal range	• Influences a loss of acidity, starch conversion into sugars, and biosynthesis of flavour volatiles.	Kader (1986)
	• A shift from aerobic to anaerobic respiration resulting in fermentative metabolism.	
	• Respiration and ethylene production rates are stimulated indicating a stress response.	

	• High CO_2 induces oxidation of ascorbic acid, possibly leading to a reduction in vitamin C levels during storage.	*Arga et al.* (1996)
	• Most recently, it was reported that low O_2 and high CO_2 atmospheres often triggers lactic acid and alcoholic fermentations leading to ethanol production.	Defillipi *et al*. (2006) Cecchini *et al.* (2011)
Optimal or sub-optimal temperature	• 'CA' can aggravate chilling injuries with temperatures below 5°C CO_2 enriched	Hess-Pierce & Kader (1984)
	• 'CA' can result in higher concentration of fermentative.	Artes *et* al. (1996).
	• Accumulation of volatiles and off flavours at a temperature above 7.5-10°C.	Defillipi *et al.* (2006)
	• Excessive weight loss due to increased transpiration.	
	• Incidences of mould due to a higher storage temperature.	
Relative humidity below or above optimal	• Low humidity causes excessive loss of weight in fruit.	Hess-Pierce & Kader, (1984), Artes *et al.*, (1996)
	• High humidity may cause mould and fungal growth in presence of high temperature.	Defillipi *et al.* (2006)

Potential benefits and adverse effects of 'CA' system

The extension of shelf life with the minimal loss of quality of horticultural produce is the most significant benefit of using the 'CA' systems. It should be noted that the efficiency of 'CA' varies, and depends on cultivar, storage temperature and gas combinations (Kader, 1980; Kader *et al.*, 1989). The 'CA' lowers respiration and transpiration rates of the product, hence minimises physiological and compositional changes (Kader *et al.*, 1986; Thomson, 1998). In

addition, a vast amount of research and literature on the benefits of 'CA' storage has been documented in literature and review papers (Brecht, 1980; Dewar, 1983; Kader, 1986; Kader *et al.*, 1988). However, Kader *et al*. (1989) and Yahia (2006) reviewed the controlled atmosphere technology, including the adverse effect. They linked 'CA' , particularly suboptimal to several adverse effects. For example, the impact on the biological stress manifested as chilling injury, wounding, induction of fermentation and accelerated decay occurs (Kader *et al.*, 1989; Yahia, 2006). These aspects tend to contribute to the development of unpleasant flavours due to the reduction in aroma biosynthesis especially when fruits are subjected to suboptimal conditions (Hess-Pierce & Kader, 2003; Defilippi *et al.*, 2006). Additional relevant information on this section, with particular reference to different empirical studies on pomegranate, has been well elaborated in Table 2.2.

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Table 2.2. Summary of the effect of 'CA' -on selected pomegranate cultivars during storage

Scope of study	Findings	References
Post-harvest physiology and storage behaviour of pomegranate fruits.	Pomegranate fruits have a low respiration rate and a non-climacteric respiratory pattern. Storage at 5 °C or lower can result in chilling injury to the fruits, and the severity of the symptoms increases with increased storage period at a lower temperature.	Elyatem & Kader (1984)
Responses of pomegranates cv. Wonderful to ethylene treatment and storage temperature	Fully ripe fruit can store for longer periods if not over-chilled. Furthermore, pomegranate does not ripen off the tree and should be picked when fully ripe to ensure their best flavour. In addition, ethylene treatments do not influence external colour, juice colour, or composition of pomegranates. Minimum safe temperatures for storage up 2 months are 3 to 5 °C and longer storage should be at 7-10 ºC	Kader *et al*. (1984)
'CA' -storage of pomegranate (*Punica granatum* L) 'Hicaz'	'CA' of (3% O_2 + 6% CO_2) preserved quality of pomegranate for 6 months at 6° C RH <95%	Kupper *et al*. (1995)
Controlled atmosphere of pomegranate 'Molar' cultivar.	'CA' of (5% O_2 + 5% CO_2) minimised loss in quality of pomegranate	Artes *et al*. (1996)

Responses of pomegranates cv. 'Wonderful' to 'CA'	Pomegranates stored at 7.5°C in 5 % O_2 + 15 % CO_2 and 90-95% RH up to 5 months free from defects and decay	Hess-Pierce & Kader (2003)
'CA' storage of pomegranate cv. 'Wonderful'	Incidence of husk scald and internal chilling injury are minimised. The effects are due to the high CO_2 level. A period above 4-month storage of shelf life was achieved for 'Wonderful' pomegranates by dipping the fruit in a fungicide and maintaining a high RH during storage. 'CA' storage at 2% O_2, + 3% CO_2 and 6–7 °C was recommended.	Nerya *et al*. (2006)
Development and control of scald to pomegranates (cv. Wonderful) during long-term storage.	'CA' with (5% O_2 +15% CO_2) decreased or prevented changes in carotenoid, acyl lipid, and phenylpropanoid metabolism that were associated with scald development in stem-end peel tissue of air-stored fruit and are indicative of stress and/or senescence	Defilippi *et al*. (2006)
Differential effects of regular and controlled atmosphere storage on the quality of three pomegranate (*Punica granatum* L.) cultivars.	'CA' with 2% O_2 + 5% CO_2 at 7 °C and regular air were investigated for 5 months. Keeping quality varied significantly, but the response in reduction of husk scalds and decay were similar. Each cultivar requires a specific protocol to maintain nutritional quality. Major phenolics decreased. 'CA' was better than RA in apparent fruit quality. However, RA maintained	Matityahu *et al.* (2016)

	anthocyanin levels in the arils and preventing occurrence of off-flavour.	
Effect of controlled atmosphere storage on pomegranate quality investigated by two-dimensional NMR correlation spectroscopy	'CA' with 5% O_2 + 15% CO_2, showed that water was transferred out of the vacuole during 'CA' storage and replaced back at a later stage of storage. This resulted in shrinkage of the vacuole and a decrease of TSS during 'CA' storage, but. aril changes were minimal in 'CA'	Zhang *et al.* (2013)

Pomegranate Industry in South Africa

In the global market where the demand for pomegranate consumption keep increasing (POMASA, 2013), it is important that postharvest handling and storage are developed to minimise losses and keep the supply chain consistent with demands in local and international markets. Many factors are contribute to the rising demand for pomegranate and eventual growth of the sector. It is imperative to note that the increase in demand for pomegranate is linked to scientific evidence of its nutritional and therapeutic properties (Al-Maiman & Ahmad, 2002; Anderson *et al.* 2014). In fact, Raymon (2011) attested to these facts in a report where he conducted a study on the marketing trends of pomegranate. He attributed the increase in demand for fresh pomegranate to the semi-processed products and nutritional and health benefits, and the ease of processing due to reduced labour. It is worth noting that an increased interest in minimally processed and fresh-cut pomegranate arils with high nutritional value has also contributed to the rising demand for consuming pomegranate (Caleb *et al.*, 2013).

Although the volumes keep growing rapidly, the southern hemisphere is still a long way to go before they can even meet the ever increasing demand levels for pomegranate fruits that are coming from the northern hemisphere during the off-season periods (POMASA, 2013). The secondary reason, yet very important is the fact that the variations in harvesting season exist between the major producing blocks located in two distinct geographic locations (the northern hemisphere and southern hemisphere) producers. The producers in the southern hemisphere include South Africa, Peru and Chile, and their harvest period are from March to May while the other block it is in season from September through February. Thus, there is a huge window of opportunity from May to September to export pomegranate to those countries in the north when the fruit can fetch an attractive price. The major importers of South African pomegranate during the off-season includes Europe, the Far East and Canada as well as other emerging African countries. With the anticipated 189% growth by the year 2017, the figures are expected to rise further to meet local and international demands (POMASA, 2013). Figure 2.1 shows the most popular pomegranate varieties grown in South Africa for local and export market. It is clear that cv. ‘Wonderful’ is the most popular variety taking nearly 50%, followed by ‘Acco’ while ‘Kessari’/’Bhagwa’ accounts for 10% (Fawole & Opara, 2013). The reason for the popularity and demand for particular cultivar stem from consumer preference. Most consumers prefer cv. ‘Wonderful’ because of its large size, relatively bigger with soft piths, larger arils and small seeds and juicier compared to other cultivars (Usanmaz *et al.*, 2014; Fawole *et al.*, 2013).

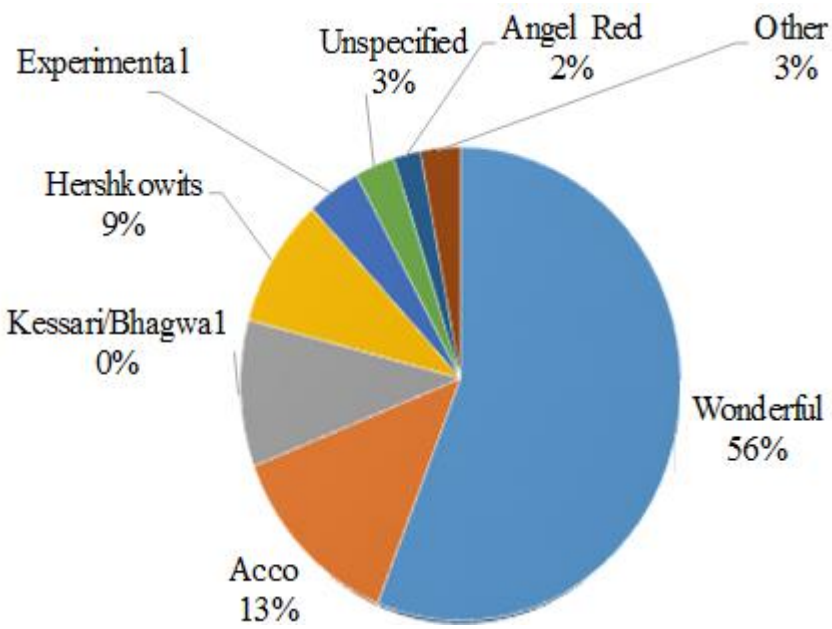

Figure 2.1. Popular pomegranate planted in South Africa (POMASA, 2013)

Pomegranate fruit, peel and arils

Pomegranate (*Punica granatum* L) is native from the Himalayas in northern India to Iran and has been cultivated since ancient times over the entire Mediterranean region (Mohammad & Kashani, 2012). Figure 2.2 shows a whole pomegranate fruit stored for 5 months under controlled atmosphere. The fruit is round, red or purple in colour and weighs about 350-500g depending on the cultivar and geographic growing conditions (Akbarpou *et al.*, 2009; Fawole *et al.*, 2013). The peel constitutes about 50% of the total fruit weight. It is an important source of bioactive compounds such as phenolics, flavonoids, ellagitannins, and proanthocyanidin compounds, minerals, mainly potassium, nitrogen, calcium, phosphorus, magnesium, and sodium, and complex polysaccharides (Ismail *et al.*, 2012). The edible part constitutes about (50%) of the fruit consists of 40% aril and 10% seeds. Arils contain 85% water, 10% total sugars, mainly fructose and glucose, and 1.5% pectin, organic acid, such as ascorbic acid, citric acid, and malic acid, and bioactive compounds such as phenolic and flavonoids, principally anthocyanins (Viuda-Martos *et al.*, 2010; Fawole & Opara, 2013). The juice is rich in polyphenol antioxidant an ellagitannin known as *punicalagin* and sugars, vitamins, minerals (Table 2.2) and several, phytochemicals and bioactive compounds) (Seeram *et al.*, 2006). Vast amounts of scientific information in literature attest the beneficial effect of bioactive compounds found in the PJ and other parts of the pomegranate fruit including the peel relevant to human health (Lansky *et al.*, 2007; Marena *et al.*, 2011). Seeram *et al.* (2006) reported in a book that pomegranate has been used in many traditions and folklore as a medicinal plant.

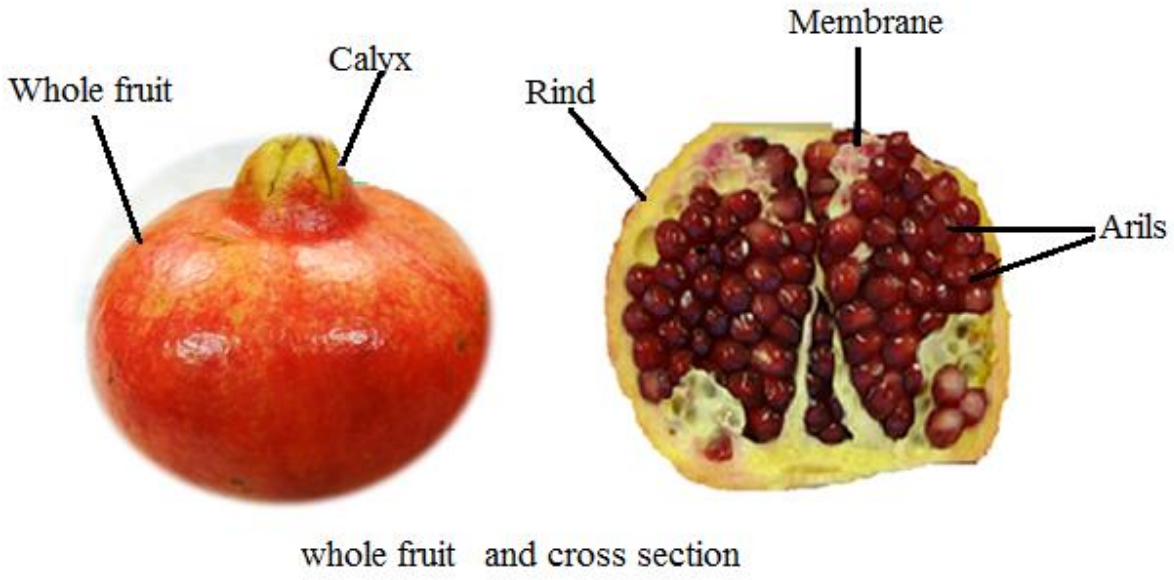

Figure 2.2. Whole pomegranate stored for 5 month under controlled atmosphere, and its section whoing internal parts

Table 2. 2. Proximate composition of fresh pomegranate

Proximate Unit		Value per100 g
Water	g	77.93 – 80.97
Energy	kcal	68 – 83
Protein	g	0.95 - 1.67
Total lipid (fat)	g	0.3 - 1.17
Ash	g	0.61
Carbohydrate, by difference	g	17 - 18.70
Fibre, total dietary	g	0.6 - 4.0
Sugars, total	g	13.67 – 16.6
Minerals(mg/100g)		
Calcium, Ca	mg	3 – 10.0
Iron, Fe	mg	0.30
Magnesium, Mg	mg	3 – 12
Phosphorus, P	mg	8 – 36
Potassium, K	mg	236 – 259
Sodium, Na	mg	3.0
Zinc, Zn	mg	0.12 – 0.35
Selenium	mg	0.6

Source: USDA National Nutrition Database (2010)

Postharvest Handling

Harvesting of pomegranate fruit stake place when fruits are fully mature usually at 130 to 180 days after the set and depend on cultivar (Holland *et al.*, 2009; Fawole *et al.*, 2013). Other maturity indices include TSS, TA and TSS/TA i.e. for 'Wonderful' pomegranate it is considered mature upon which the TSS reaches 17-18% and titratable acidity of 1.58–1.8% the fruit is considered ready for harvest (Kader *et al.*, 1984). The common practice for harvesting is manual hand picking, followed by assembling at grading plate and packing in cartons /boxes. Parasad *et al.* (2010) highlighted the importance of the process of harvesting to ensure as minimal physical damage as possible to enhance the longer shelf life of pomegranate. Opara & Pathare (2013) emphasised the consequences of bruise damage during all stages of postharvest handling especially during packhouse operations, transport and storage, and how they contribute to postharvest losses of fresh horticultural produce. It is widely

reported that failure to control the most critical limiting factors such storage temperature, bruising and general postharvest handling practices could precipitate severe physiological disorders (Hess-Pierce & Kader 2003; Roy & Wasker, 2005; Opara & Pathare, 2013). Some important physiological and compositional changes have been discussed in detail in the preceding sections.

CURRENT STORAGE PRACTICE

The modified atmosphere (MAP) packaging is the popular method used for packaging and storage of pomegrate in cold atmosphere and transportation (Kader *et al.*, 1989). The technique involves the use of polymeric films with a wider range of gas – diffusion properties, the most common being Xtend® film packaging bags (StePac, Tefen, Israel) (Zagory & Kader, 1988). The Xtend® film bags have had a remarkable success record of minimising weight loss (Nanda *et al.*, 2001), water loss, and /or atmospheric modification of O_2, CO_2, and C_2H_2 (Kader, 1989). However, no further control is exerted over the initial gas composition, thus a gas composition in MAP is likely to change with time owing to the respiration, diffusion of gases into and out of the product (Artes *et.al.* 2000; Nanda *et al.*, 2001; Porat *et al.*, 2008). The notable limitation of the MAP includes a possible build-up of CO_2, which can cause anaerobic fermentation, and reduction of related compositional properties including shorter postharvest life less than eight weeks (Kader *et al.*, 1989; Artes *et al.*, 2000). For example, 'Mollar de Elche' pomegranates (*Punica granatum* L.) was stored at 2-5 °C for 12 weeks in unperforated polypropylene (UPP) film of 25 µm thickness at 5 °C, its final total anthocyanin content decreased at the end of shelf life while water loss and chilling injuries were minimized and without incidence of decay (Artés *et al.*, 2000). The characteristic of Xtend® film bags is that of lowering excessive loss of moisture through the film that acts a barrier for water vapour transmission through the package. Table 2.3 shows some vital characteristics of MAP packaging films for pomegranate and other products on the market.

Table 2-3. Gas permeability properties of some films available for packaging fresh produce(Zagory & Kader, 1988)

	Permeability cc/m^2/mL.day at 1 tm		Gas ratios
Film type	CO_2	O_2	CO_2/O_2
Polyethylene: Low density	7,700 - 77,000	3,900 - 13,0000	2.0 - 5.9
Polyvinyl chloride	4,263 - 8,138	620 - 2,248	3.6 - 6.9
Polypropylene	77,700 - 21,000	1,300 - 6,4000	3.3 - 5.9
polystyrene	10,000 - 26,000	2,600 - 7,7000	3.4 - 3.8
Saran	52 -150	8 - 26	5.8 – 6.5
Polyester	180 – 390	52-130	3.0 - 3.5

PHYSIOLOGY OF POMEGRANATE

The physiological behaviour of pomegranate is that of a non-climacteric pattern which exhibits the very low respiratory pattern. They produced trace amounts of C_2H_4 with no significant response to exogenous C_2H_4 treatments as measured by changes in skin colour, and juice colour and composition (Elyatem & Kader, 1984). The notable physiological behaviour is provided in the next section.

Respiration and ethylene production

Respiration is the process by which organic materials (carbohydrates, proteins, and fats) are broken down into simple products with a release of energy. Oxygen (O_2) is used in this process and carbon dioxide (CO_2), water and energy are produced. The respiration rate (RR) is temperature dependent, increases with temperature, the higher the temperature, the higher the RR, the faster the deterioration rate and shorter the postharvest life of any given commodity. In the case of pomegranate fruits, it exhibits low RR, which declines during storage (Elyatem & Kader, 1984; Kader *et al.*, 1984). At a lower temperature, 5 °C, the RR is 8 mL /kg- hr of CO_2 during storage (Elyatem & Kader, 1984).

Table 2.4 shows a pattern of both respiration rate and ethylene production. Ethylene (C_2H_2) is the simplest form of organic compounds affecting the physiological processes such as senescence (ripening) of fruits. It is produced by all tissues of higher plants and by some microorganisms. In pomegranate, very low amount of ethylene below 0.2 (microliter per kg/hour) is produced at 20 °C and less than 0.1 (micro litre/kg.h at 10 °C (Cristol *et al.,* 1989).

Table 2.4. Effect of temperature on respiration rate and ethylene production of pomegranate (Cristol *et al.,* 1989).

Storage temperature	Temperature	5°C	10°C	20°C
Rate of respiration	ml CO_2/kg.·h	2-4	4-8	8-18
Rate of ethylene production	µl/kg·h	<0.1	<0.1	<0.2

Chilling Injury

Chilling injury (CI) is a physiological disorder that occurs in fresh fruits stored below 5 °C. The external symptoms include brown discoloration of the skin and increased susceptibility to decay (Elyatem & Kader, 1984). Internal symptoms include a pale colour of the arils (pulp around the seeds) and brown discoloration of the white segments separating the arils (Elyatem & Kader, 1984). The symptoms become more visible when the fruit is transferred to 20 ˚C for three days (Artes *et.al.,* 1998; Defillippi *et al.,* 2006; Palour *et al.* 2007). Studies have suggested that CI can be minimised when storage temperature is above 7 ˚C (Kader *et al.,* 1984; Defillipi *et al.,* 2006).

Weight loss

Weight loss in pomegranate is one of the major limiting factors to prolonged storage of the fruit (Ben-Arie & Or, 1986). The primary factors that contribute to weight loss include high storage temperatures, low relative humidity and poor handling practices of the produce. As fruits lose moisture through transpiration, the immediate economic effect is the reduction of saleable weight especially when weight loss exceeds 5% (Ben-Yehoshua & Rodov, 2003; Mahajan *et al.,* 2009). The symptoms are wide but the common once includes fruit wilting and/or shrivelling which subsequently leads to loss of appearance, quality, shelf life and profitably. Methods that can prevent or lower the

rate of moisture loss include low-temperature storage practices, modified atmospheric packaging and waxing (Artes *et al.*, 2000) (Hess-Pierce & Kader, 2003). Recently, studies involving 'CA' have shown significant strides in reducing moisture by lowering respiration and transpiration (Kupper *et al.*, 1995; Pierce-Hess & Kader, 2003; Nerya *et al.*, 2006; Defillipi *et al.*, 2006). In addition, optimal storage temperature and RH between 85-95% have the potential to minimise respiration and transpiration rate and ultimately reduce weight loss (Kupper *et al.*, 1995; Artes *et al.*, 1996; Pierce-Hess & Kader, 2003; Defillipi *et al.*, 2006; Nerya *et al.*, 2006).

Husk Scald

Husk scalds are characterised by browning or discoloration of the husk (without any internal symptoms on the arils or surrounding tissues) that occurs during storage for more than three months at 7°C or lower temperatures (Defillipi *et al.*, 2006). The delay in harvest has the potential to increase susceptibility to scalds in pomegranate (Hess-Pierce & Kader, 2003).

COMPOSITIONAL CHANGES

Many physiological and chemical changes take place during development and maturation of the fruits. Some may continue after harvest and can be desirable or undesirable (Kader, 2006). In pomegranate, development of anthocyanin (red and blue colours) is desirable in fruits to give an attractive and signal beginning of maturity. Changes in anthocyanin and other phenolic compounds, however, are undesirable because they may result in tissue browning (Kader, 2011). Changes in organic acids, proteins, amino acids, and lipids can influence flavour quality of the commodity. The loss in vitamin content, especially ascorbic acid (vitamin C), is detrimental to nutritional quality (Kader & Yahia, 2011). Production of flavour volatiles associated with ripening of fruits is very important to their eating quality. In consideration with pomegranate, the fruits contain 70-90% water, and once separated from the source of nutrient (plant) they tend to accelerate the respiration, transpiration resulting in many compositional changes due to catabolic process (Barrett, 2006). Although pomegranate is non-climacteric fruit with no expectation of senescence after harvest, compositional changes occur when storage environment is not optimal (Hess-Pierce & Kader, 2003). Fawole & Opara (2013) investigated the compositional changes pomegranate fruit 'Bhagwa' and 'Ruby' at stages of maturity with particular interest on TSS, pH, titratable acidity (TA) among others quality parameters. The authors reported that cv. 'Bhagwa' the TSS had a TSS of 16.18 ºBrix at a full ripe stage, whereas the TA was 0.28 %. In the case of 'Rubby,' the TSS was 15.06 ºBrix whereas the TA was 0.38%. These findings highlight variation in compositional changes between cultivars at the

same stage of optimal maturity. During storage, further changes occurred depending on the storage condition. For example, under different 'CA' storage system, the compositional changes are lower than cold air and the rate depends on several factors, such as the relative humidity, temperature, cultivar and duration (Kupper *et al.,* 1995; Artes *et al.,* 1996; Defillipi *et al.,* 2006).

Total soluble solids (TSS)

The total soluble solids (TSS) are solids that are dissolved within a substance, in case fruits, its refers mainly to sugars. It is a vital quality attribute used as one of the maturity indices in determining the optimal ripeness of pomegranate. The total soluble solids vary considerably among cultivars ranging from 15.2-22.0 °Brix (Akbarpour *et al.* 2009; Tehranifar *et al.* 2010). The South African pomegranate cultivars reported by Fawole & Opara (2013), 'Rubby' had 16.18 while 'Bhagwa' had 15.06 ˚Brix. In addition, *Chace at al*. (1981) reported 17 ºBrix for cv. 'Wonderful' pomegranate. However, after harvest, and during storage, studies have shown significant changes in TSS depending on the storage regime. For examples, Hess-Pierce & Kader (1984) observed a reduction in TSS for cv. 'Wonderful' during the five months' storage period. Similarly, Kupper *et al.* (1995) observed a reduction in TSS in 'Hicaz' stored in different 'CA' combinations. The authors gave no scientific reason; yet, Zhang & McCarthy (2013) in his work attributed the compositional changes in TSS to the migration of water out of the vacuole and/or back to the vacuole in the later stage of storage. Such biochemical changes influenced or resulted into fluctuation trends of TSS during storage. This hypothesis corroborated with the argument reported on TSS for Mangoes. The changes were attributed to enzymatic conversion of organic acids to sugars through gluconeogenesis and lowering moisture content in fruits during storage (Echeverria & Valich, 1989).

Total acidity (TA) and pH

Titratable acidity (TA) expressed, as citric acid is an important quality parameter in pomegranate because it contributes to sour taste. Citric and malic acids are predominant in the majority of pomegranate cultivars, but in some cultivars, large amounts of oxalic and tartaric acids were detected. In those varieties, only one had oxalic acid as the major organic acid (Miguel *et al.* 2004). It is evident that TA varies among cultivars and/or depends on the stage of maturity and growing region (Fawole *et al.* 2011; Fawole *et al.* 2013). I. e. 'Shlefy' grown in the North- East of Libya harvested at optimal maturity stage had a TA level of 1.5 mg /L (Ghafir *et al.,* 2010), whereas pomegranate grown in Iran had the range of TA from 0.35 mg - 3.36 mg/L (Akbarpour *et al.,* 2009). Depending on the storage method, either TA can increase or decrease as was reported under cold storage at 5 °C compared to room air at 20 °C (Hess-Pierce & Kader 1984). Under the 'CA' storage regime, as it effectively

lowers the respiration rates the atmosphere also retards several compositional changes linked to the action of enzymes aconitase, isocitrate dehydrogenase in the Krebs cycle (Kader, 2006). Given that the effect of 'CA' response to pomegranate varies, it highly probable that the resultant effect on compositional changes would also vary and produce different results. The two quality attributes, TSS and TA are responsible for the ratio of (sugar to the acid) (TSS: TA) which accounts for 'sweet' and 'sour'' taste sensations (Mayuoni-kirshinbaum *et al.* 2012).

Volatile compounds

Pomegranate is a good source of valuable volatiles and flavour compounds. The aroma volatile compounds in freshly harvested pomegranate cultivars have been characterised by researchers (Vazquez-Araujo *et al.*, 2010; Calın-Sanchez *et al.*, 2010; Mayuoni-Kirshinbaum et al., 2012). The notable aroma volatiles groups in fresh pomegranate (Carlin-Sachez *et al.*, 2011), range from 18-22 volatiles compounds grouped as follows (alcohol, aldehydes, ketones, monoterpenes, oxygenated monoterpenes, sesquiterpenes and esters) (Carlin-Sachez *et al.*, 2011; Mayuoni-kirshinbaum *et al.*, 2012). The quality of fresh fruits can be defined in terms of factors such as appearance, firmness, colour, flavour, and nutritional value. Controlled atmosphere ('CA') has been studied and known to reduce the incidence of decay and to preserve quality attributes (Kader *et al.*, 2006). However, not all quality characteristics can be preserved to the same extent. Flavour and volatile compounds have not been reported, even though studies on other fruits have shown that it tends to decline before prior to any changes in appearance (Kader *et al.*, 2006). Thus, Kader (2008) recommended that postharvest life of fruit should be determined based on flavour quality than appearance. In light of that recommendation, Mayuoni-Kirshinbaum *et al.* (2012 evaluated the impact of MAP on the changes in aroma volatile composition during prolonged storage of cv. 'Wonderful' pomegranate. The results showed that changes in volatiles composition occurred with storage duration. A steady increase in ethanol and aldehyde groups developed which influenced sensory properties of pomegranate. Although 'CA' has proven successful in extending the shelf life of pomegranate, no information exists on its impact on flavour and volatile composition of pomegranate cultivars (Caleb *et al.* 2013). It seems evident that lack of information on the influence of 'CA' on pomegranate merits further investigations.

Colour

Pomegranate fruit derives its red colour from the natural pigment called anthocyanin (cyanidin, delphinidin, and pelargonidin) (Ozgen *et al.*, 2008). Consumer's preference in liking or disliking any fruit begins with and/or depends on a mixture of quality attributes such as rind colour, sugar content,

acidity, and flavour (Al-Said *et al.*, 2009). The stability or any possible change in the physiological quality of pomegranate have been widely studied (Holcroft *et al.*, 1998). In this study, it was reported that CO_2 concentration not exceeding a moderate 10% CO_2 had potential to minimise the loss of external colour and for the arils. They concluded that CO_2 did not affect anthocyanin in pomegranate. Artes *et al.* (1998) investigated unique treatment involving intermittent warming followed by storage at 0 °C and/or 'CA' . Results showed no significant changes in colours of pomegranate fruit. They consequently, concluded that optimal 'CA' could enhance or preserve the external colour of pomegranate and other horticultural products. However, with scanty reports showing the negative influence of enhanced CO_2 as reported by Kupper *et al.* (1995), it can be concluded that changes in the colour of pomegranate depend on several factors including cultivar, storage temperature and storage duration, hence refinement of 'CA' should be conducted for individual cultivars.

FUTURE PROSPECTS

The future prospect of commercial application of 'CA' relies on its success in reducing the postharvest loss of pomegranate through lowering physiological and compositional changes induced by (e.g. respiration and transpiration rates) during storage. Kader (2003) reported that since 1997 there have been a few modest increases in the commercial use of 'CA' during transport of several commodities in the world. 'CA' storage permits the harvested fruit keep longer at optimal temperatures and relative humidity. In addition, 'CA' has the potential to keep economic fundamentals at a profitable level. i.e. (market price, quality, supply and demand) which are primarily determined by the postharvest technology used. It is evident that the critical factors that limit shelf life of pomegranate (weight loss and Shrinkage) have shown prospects of being lowered by an optimal 'CA' technology (Ben-Arie & Or, 1896; Kupper *et al.*, 1995; Artes *et al.*, 1996; Elytem & Kader, 2003; Defillipi *et al.*, 2006). The end of flavour life of pomegranate results from losses in sugars, acids and aroma volatiles (esters) and/or development of off-flavours (due to fermentative metabolism). The possible role of 'CA' in delaying these undesirable changes should be investigated and optimised. Based on this extensive review, it is highly probable that 'CA' will continue to thrive as the best choice for postharvest storage of pomegranate.

CONCLUSIONS

This review has shown that 'CA' is a robust and evolving storage technology with numerous potential benefits compared to room air cold storage conditions. The ability to extend shelf life two-four fold more after harvest places 'CA' on a competitive bid to satisfy the demands for long terms export market expectation during the off-season periods. More significantly, the reduction of weight loss,

physiological and compositional changes during the supply chain are considered an important contribution to a consistent supply of fruit with high nutritional benefits. However, 'CA' per se has not decisively addressed all nutritional and quality concerns. The gaps in knowledge of the impact of 'CA' on important bioactive and volatile compounds need further investigations. Accordingly, the foreseeable challenges that lay ahead in the use of 'CA' are based on the complexity in the standardising/holistic optimisation of the technology. Reasons are based on for the variation in pomegranate response to 'CA' conditions includes the genetic make-up, geographic location where fruits are grown, and the development of new cultivars favoured by consumers. The cost of investment in 'CA' technology and skill to operate are considered as hurdles to investing in 'CA' . Despite these alternate views, 'CA' remains a viable technology for use in the long term.

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CHAPTER 3

RESPONSE OF WHOLE POMEGRANATES ('WONDERFUL' AND 'BHAGWA') TO CONTROLLED ATMOSPHERE CONDITIONS

Abstract

Pomegranate fruits ('Wonderful' and 'Bhagwa') were stored in two different controlled atmosphere storage conditions (CA1: 3% O_2 + 6% CO_2, CA2: 5% O_2 + 14% CO_2) and one type of Xtend® film packaging at two different temperatures 5 and 20 °C and 95% relative humidity. Evaluations of physiological and quality responses were done at monthly intervals for 6 months. The results of this study showed that, changes in physical and chemical properties were influenced by storage temperature, duration and largely by the 'CA' conditions. Storing fruits under CAs at RA= 5 °C preserved the quality and extended the shelf life of fruit to 4 and 5 months for pomegranate fruit cv. 'Bhagwa' and 'Wonderful', respectively. The CA2 with 5% O_2 + 14% CO_2 fairly preserved total soluble solids / titratable acid ratio with negligible weight loss compared to the other treatments. Fruits stored in Xtend® film packaging had likewise insignificant change in weight loss and extended shelf life for three months more than in room air at 5 °C. Overall, results revealed the superiority of the CA2 condition among other treatments in reducing weight loss and maintaining most of the quality attributes at reasonably higher level.

Introduction

Pomegranate fruit (*Punica granatum L.*) is a non-climacteric fruit (Elyatem & Kader, 1984), and can grow in tropical and temperate regions (Johnson, 2002; Jalikop, 2010). The fruit is rich in antioxidants, pharmacological and therapeutic related constituents such as ant-carcinogenic and anti-inflammatory properties (Lansky & Newman, 2007; Jurenka, 2008). The health quality attributes have boosted global demand and export for pomegranate fruit which is now being consumed fresh and/or processed into various industrial products (Opara & Al-ani, 2009; Viuda-Martos *et al.*, 2010). In terms of postharvest, pomegranate fruit is considered highly perishable with a limited shelf life of two months under room air storage at 5 ^{o}C (Elyatem & Kader, 1984; Artés *et al.*, 1996). The major problems being weight loss, chilling injury, high incidence of moulds and decay affecting overall fruit quality during storage (Defilippi *et al.*, 2006; Mirdehghan *et al.*, 2007).

In order to sustain a continued global supply of pomegranate, 'CA' storage has been investigated as an alternative to chemical storage methods such as diphenylamine (DPA) and/or 1-methylcyclopropene (1-MCP) and waxing (Artés *et al.*,1998; EC, 2012). 'CA' operates by lowering

the respiration and metabolic processes as well as delay senescence (Mahajan & Go Swamii, 2004; Kader & Yahia, 2012). Empirical studies for different 'CA' with gas composition and optimal temperatures extended shelf life of pomegranate for 5 months depending on cultivars (Kupper *et al.*, 1995; Artés *et al.*, 1996; Defilippi *et al.*, 2006). In South Africa, there is ample scientific information on the commercial use of 'CA' storage for deciduous fruits such as apples and pears (Eksteen & Truter, 1986). To the best of knowledge, there is limited scientific information on the commercial application of 'CA' on pomegranate grown in South Africa.

Currently, the use of Xtend® film packaging of pomegranate offer a temporal storage and handling of pomegranate among the South African pomegranate industry even though not much has been documented regarding its effectiveness in maintaining quality during storage. Therefore, the objectives of this study were to investigate the physiological and quality response of two selected pomegranate cultivars to two 'CA' treatments. In addition, to evaluate the effect of Xtend® film package on quality of pomegranate during storage at different storage temperatures.

Material and Methods

Materials

Pomegranate fruit 'Wonderful' and 'Bhagwa' grown in South Africa were procured from a packhouse located in Wellington, Western Cape (33.63° 'S, 18.98° 'E). At the packhouse, fruits were sorted to remove defective fruit, cleaned with 1% chlorinated water followed by fruit samples surface removal, which would otherwise create condensation in the Xtend® film after package (StePac, Tefen, Israel) and placed in cardboard boxes, containing 12-18 fruits each. Sixty boxes were transported to the Postharvest Research Laboratory at Stellenbosch University, stored at 5 °C, and 90% RH for 24 hours before commencing the storage experiments.

Experimental setup

The experimental setup consisted of two different 'CA' atmospheres (CA1: 3% O_2 + 6% CO_2 and CA2: 5% O_2 + 14% CO_2). The samples were removed from the Xtend® film package followed by storage in the 'CA' cabinets at 5 °C and 95% RH. 'CA' cabinets had a total volume of 100 cm^3, and designed with tubes delivering a continuous flow of gas mixture (± 0.5%). Xtend® film packaging bags containing fruits were placed in the cardboard boxes and stored at 5 and 20 °C, referred to as XT5 and XT20, respectively. The control fruits were taken out of the Xtend® film package and kept in an open air as opposed to closed bags during storage at 5 and 20 °C referred to as RA5 and RA20, respectively. Data loggers to monitor temperature and RH were placed in 'CA' cabinets and in room

air within the cold rooms. Gas composition inside the Xtend® film package was measured using Checkmate gas analyser (PBI Dansensor, Ringsted, Denmark). The entire experiment was replicated twice with six fruits analysed per sampling day per treatment to evaluate physical and chemical analysis over a six-month period.

Respiration rate

The rates of oxygen consumption (RO_2) and of carbon dioxide evolution (RCO_2) of whole pomegranate fruit were measured using a closed system method (Caleb *et al.*, 2012) at the beginning and the end of 'CA' storage (six months), following a three day storage in room air (21% O_2 + 0.03% CO_2). Individual fruits, 351 g 'Wonderful', and 231 g 'Bhagwa', respectively, were put in glass jars in triplicate and kept at temperatures 5, 7.5 and 10 °C for 72 hours to acclimatise. Petroleum Jelly was incorporated into the gap between the lid and jar for all the glass jars in order to ensure no leakage of gas from the glass jar. The O_2 and CO_2 gases were monitored hourly over a four-hour period using a gas analyser (Checkmate 3, PBI Dan sensor, Ringsted, Denmark). Respiration rate of the whole fruit in terms of oxygen consumption (RO_2) and carbon dioxide production (RCO_2) was quantified by equations (1 and 2) in triplicate.

$$y_{O_2} = y_{O_2}^{i} - \frac{R_{O_2}\ .W}{V_f} x\ (t - t_i)\ x\ 100 \qquad (1)$$

$$y_{CO_2} = y_{CO_2}^{i} + \frac{R_{CO_2}\ .W}{V_f} x\ (t - t_i)\ x\ 100 \qquad (2)$$

Where, $y^{i}_{O_{2,}}$, $y^{i}_{CO_2'}\, y_{O_{2,}}$, $y_{CO_2} y^{i}_{O_{2,}}$, $y^{i}_{CO_2'}\, y_{O_{2,}}$, y_{CO_2} are, respectively, the O_2 and CO_2 concentrations in volumetric fraction in the gas mixture at the initial time ti (h) (or time zero) and at time t (h). RO_2 and RCO_2 are the respiration rates (mL CO_2/ (kg.h) and W is the weight of the pomegranate fruit (kg) and V_f is the free volume inside the package (mL).

Physiological disorder

The whole pomegranate fruits were visually inspected for external physiological disorders and cut to examine internally for any form of decay as described by Palou *et al.* (2007). The physiological disorders (skin pitching, Mould, scalds and decay) were quantified using a 3-point scale, 0 = none visible; 1 = slight (≤ 25% of the skin); 2 = moderate (26 - 50% of the skin); and 3 = severe (> 50% of the skin) while fruits with incidence of mould were automatically regarded as spoiled. Treatments in which more than 25% of the stored fruit displayed decay were terminated.

Weight loss

Three pomegranate fruits from each of the five boxes per treatment (total 15) were labelled and their weights were monitored throughout the storage period. Weight loss was expressed as a percentage of initial weight.

Total soluble solids (TSS), titratable (TA) and pH

The arils from six pomegranates per treatment were separately blended and seeds separated from juice from which the TSS, TA, and pH were measured using standard methods. Total soluble solids were measured at 20ºC using a digital refractometer (Abbe refractometer model 10450, American Optical, Buffalo, NY), and TA was determined potentiometrically using four grams of juice diluted with 20 mL of distilled water and then titrated to an end point of 8.1 - 8.2 using 0.1N NaOH and expressed as percentage of citric acid. The pH was measured at room air by a pH meter (Model 507 Crimson, Barcelona, Spain). All samples were done in duplicate and results expressed as mean standard error (± SE).

Colour measurement

Colour measurement of whole pomegranate fruit was done using a method described by Holcroft *et al*, (1998). The procedure involved taking three fruits per treatment, label and measure the colour parameters *L**, *a**, *b** along the four equatorial points of each fruit using colorimeter (Model CR-200B Handheld, Minolta, Arizona).(Holcroft *et al*, 1998). The Chroma C* value and hue angle h° were calculated according to equations 3 and 4 (Al-said *et al.*, 2009; Pathare *et al.*, 2012).

$$C^* = (a^2 + b^2)^{1/2} C^* = (a^2 + b^2)^{1/2} \quad (3)$$

$$h^* = \arctan\left(\frac{b}{a}\right) h^* = \arctan\left(\frac{b}{a}\right) \quad (4)$$

Where; a* and b*, are the colour values of the pomegranate skin

Textural properties

The fruit firmness of the whole pomegranate fruit was measured in terms of puncture resistance force using a texture profile analyser (Model TA.XT2; Stable Micro Systems, UK), with a 75 mm compression probe. The probe was programmed to penetrate 10 mm into the test fruits with a speed of 10-mm. s^{-1}. Duplicate puncture tests were performed on the opposite sides of the equatorial region of each fruit. Peak force required to puncture external fruit skin was taken as firmness resistance in

(N). Five fruit were randomly chosen from each experimental batch and used and results were calculated as the means ± S.E. A single aril compression test was performed using a texture profile analyser with 10 kg loading cell and a cylindrical compression probe with a diameter of 35 mm. The instrument was pre-set at a speed 1.5 mm. s^{-1}, 0.5 mm. s^{-1} test speed, 10.0 mm. s^{-1} post-test speed, and 0.20 N trigger forces. Hardness, as expressed in Newton (N), was obtained as means of 20 replicates (Fawole *et al.*, 2011).

Data analysis

One-way analysis of variance (ANOVA) at 95% confidence interval was applied to analysed data. All experiments were carried out in triplicate and data were analysed using Statistical software (Statistica 10.0, Statsoft, USA).

RESULTS AND DISCUSSION

Gas composition

Changes in gas composition in Xtend® film bags stored at 20 and 5 °C are presented (Figure 3. 1a, b). Overall, the changes in gas compositions (CO_2 and O_2) were temperature dependent. The lower the temperature (XT5) the lower the respiration rate and vice versa for fruits stored at 20 C, in XT 20, which exhibited high respiration rate. Thus, an increase in CO_2 from 0.03 to 14.3% was observed by the end one-month period, a subsequently increased by 5% by the second months for 'Wonderful' stored in XT20 at room air. This experiment was terminated due to a higher percentage of fruit decay and general physiological disorder (decay). The changes in XT5 bags were comparatively low (3.4% CO_2) in the first month and later increased to 5.8% by the end storage period for'Wonderful'. A similar trend in gas changes was observed for 'Bhagwa' stored in XT20 packaging bag with an increase in CO_2 from 0.03 to 9.9% in the same period. None of the Xtend® film bags effectively maintained the gas composition similar to optimal levels such as (5% O_2 + 5% CO_2) or (5% O_2 + 15% CO_2) reported as certain pomegranate cultivars 'Molar' and 'Wonderful', respectively (Artes *et al.* 1996; Hess-Pierce & Kader, 2003; Defillipi *et al.* 2006). In addition, Nanda *et al*. (2001) proposed specs for shrink films with properties such as WVTR (4.43 x 10-8 g/M^2 /day unit) and gas permeability rate of 2.23 x 10-8 CO_2 and 5.57 x 10-19 (mol S^{-1} $M.m^2$. Pa^{-1}) potentially suitable for maintaining the quality of cv. 'Ganesh' pomegranate longer than in room air.

Respiration rate

The respiration rate in terms O_2 consumption (RO_2) and evolution of CO_2 (RCO_2), respectively, are presented on (Figure 3.2a, b) for 'Wonderful' and (Figure 3.2a, b) for 'Bhagwa' pomegranate. The initial RO_2 for cv. 'Wonderful' ranged from 5.4 to 16.7 O_2 mL / (kg. h), and CO_2 production rate within 5 to 17 CO_2 mL / (kg. h) across the test temperatures 5, 7.5, and 10 °C presented in (Figure 3.3a, b). A similar pattern was observed in RO_2 and RCO_2 for pomegranate 'Bhagwa' (Figure 3a, b). The respiration rate (RR) of fruit stored in CA1 followed by three-day storage in room air showed a significant variation in the pattern when compared CA2. Fruits stored in CA1 (3% O_2 + 6% CO_2) showed slightly higher rate compared the baseline values. Similarly, fruits stored in CA2 showed a significantly ($p<0.05$) higher rate than the CA1 and baseline values. The influence of temperatures (5, 7.5 and 10 °C) was noticeably seen to influence the RR with values increasing with temperature from 9.6 mL/ (kg. h) CO_2 at 5 °C to 38.7 mL/ (kg. h) at 7.5 °C and 38.9 mL/ (kg. h) at 10 °C for fruit stored in CA2 with (5% O_2 +14% CO_2. The results of this study agree with those reported for Strawberries fruits (Li & Kader 1989). The authors investigated the effect of 'CA' storage of strawberries with relatively low O_2 and high CO_2 conditions. It was observed that RR fluctuated by an increase and/or decrease in respiration rates during recovery process after exposure to 'CA' which caused stress. The change in RR had an influence on respiration quotients (RQ10) showing a slight increase signifying a shift from catabolism of carbohydrate to organic acids (Elyatem & Kader, 1984; Fonseca *et al.*, 2002). Furthermore, Kader & Ben-Yehoshua (2011) reported similar results in horticultural produce and attributed the change in respiration rate the exposure of fruits to low and /or high CO_2.

Physiological disorder

The effect of storage conditions and duration on the physiology of pomegranate is presented on (Figures 3 4a, b). It was observed that incidences of disorder (mould and decay) increased with storage temperature and duration. Storage temperature in RA at 20 °C resulted in high percentage disorder (26% room air) and (41.6%) in Xtend® film bags during the first months thus prompted the termination of an experiment as recommended by Palou *et al.* (2007). As storage duration progressed fruit decay in Xtend® film bags in XT5 increased to 6.6% by the end of storage period.

There was no fruit decay under 'CA' in the first three months, but as storage period progressed to four and five months incidences of decay emerged to less than 2% under CA2 compared to CA1 for 'Wonderful' pomegranate. The pattern of fruit decay for 'Bhagwa' cultivar (Figure 3.4a, b) was

similar to Wonderful' pomegranate although the magnitude of decay were slightly higher, indicating differences in cultivar response.

The physiological response of fruit stored under respective storage conditions showed an interaction between treatments 'CA' with temperature. The temperature had a significant ($p<0.05$) influence on decay showing relatively higher incidences in RA at (20 °C) temperature than at 5 °C under 'CA' . These results agree with those reported in the literature (Arts *et al.*, 2000; Nanda *et al.*, 2001). Lower temperature (5 °C) and the use of Xtend® film bags at XT5 had a positive influence in reducing physiological disorder (decay). The results of this study corroborate with those reported by Nanda *et al.* (2001) that shrink films have potential to lower transpiration and minimise weight loss, fruit shrinkage, scald development, and decay compared with room air. However, the Xtend® film bags used by the South African pomegranate growers /industry appeared to have poor gas retention properties as observed and reported on (Figure 3.4, a, b).

Overall, physiological response of pomegranate under 'CA' conditions studied corroborated with literature information prolonging the shelf life with minimal disorders (Kader *et al.* 1995; Hess-Pierce & Kader, 2003; Defilippi *et al.*, 2006). These authors attributed the non-occurrence of mould and other physiological disorders in respective 'CA' atmosphere to higher CO_2 that provided the anti-fungistatic properties. By comparison, it was evident that treatment and temperature had an influence of quality attributes as evident from low storage temperature and 'CA' with 14% CO_2 showing remarked protection of disorder better than 'CA' with 6% CO_2 atmosphere.

Weight loss

Weight loss (%) during storage is presented in (Figure 3. 5a, b). Storage temperature and duration had an impact on weight loss, where fruits stored at 20 °C in the room air condition had higher percentage weight loss than 5 °C in air. Also at the same temperature 5 °C, fruits stored in Xtend® film and 'CA' had a lower percentage weight loss than those stored in open room air. The loss of weight gradually increased with storage temperature and duration of storage, with the highest loss at 20 °C on last day of storage in the room air condition. Fruit stored at 5 °C showed a minimal increase from zero to 7.3% in room air, and less than 1% in fruit stored under XT5, 'CA' in the first month. As with a passage of storage period weight loss room air at 5 °C increased from 9 to 17% in the 2nd and 5 months of storage. On the other hand, fruits stored in (XT5 and 'CA') had minimal loss in weight ranging below 5% at the end of five-month storage period. Figure 5b 'Bhagwa') showed a similar trend in percent weight loss which increased with temperature and duration of storage. The results corroborate with those reported by Artes *et al.* (2000) and Nanda *et al.* (2001). The reduction in weight loss with minimal loss in quality exhibited by Xtend® film bags corroborate with findings

reported by Al-Mughrabi *et al.* (1995) that storage temperature and duration has a significant impact on weight loss.

Under 'CA' conditions, particularly CA2 (5% O_2 + 14% CO_2) was more effective in reducing weight loss compared to the rest of the treatments. These results corroborate positively well with earlier literature reports (Kupper *et al.,* 1995; Nerya *et al.,* 2006), where pomegranate 'Hicaz' and 'Wonderful', respectively, had superior quality over fruits stored under room air. Storage temperature had a significant influence on compositional changes showing a linear relationship. As temperature increased 5 to 7.5 °C under CA2, TSS increased by 21%, whereas at 5 °C under CA2 a reduction by 1.9% was observed. The phenomenon was well elaborated by Zhang & McCarthy (2013). In summary, and using GLM model, it was observed that TSS of fruit stored under CA1 at 5 & 7.5 °C showed no significant difference in the response with respect of TSS implying that temperature was not a major influencing factor.

Fruit quality characteristics

Total soluble solids (TSS)

Changes in total soluble solids (TSS) during storage are shown in (Figure 3.6 a, b). The TSS decreased by 1-2 °Brix from an initial mean 17.5 °Brix for 'Wonderful' and 15°Brix for'Bhagwa,' respectively. Storage temperature and conditions had an impact on the changes in total soluble solids. Reduced TSS was observed with time in both CA1 and CA2 stored pomegranate, but there was no clear difference between the two storage treatments (Figure 3.6). The low storage temperature at 5 °C resulted in low TSS (15.7°Brix) compared to (17.0 °Brix) observed at 20 °C for cv. 'Wonderful' during the two-month storage period. As storage duration progressed, a decrease and/or increase in TSS was observed across all storage conditions at 5 °C which corroborates with findings reported by Kader *et al.* (1984) and (Al-mughrabi & Bacha. (1995). Nanda *et al.* (2001) reported similar changes in TSS for pomegranate stored under MAP. Changes in TSS under CA2 storage conditions showed a slight decrease of 8% compared with the value at baseline (17 °Brix) for cv. 'Wonderful' pomegranate. The influence of temperature and treatment (CA1 or CA2) was significant ($P<0.05$) showing a slightly higher value at 7.5 °C under CA2) (Figure 3.11). The findings of this study are in agreement with the previous studies reported by several researchers who studied different pomegranate cultivars (Kupper *et al.*, 1995; Kader, 2003). Zhang & McCarthy (2013) explained the cause of compositional changes showing a slight decline in TSS, or in some cases fluctuated during the storage period. They attributed to the migration process of water in and out of the vacuole at an early stage and/or back to the vacuole in the later stage of storage.

Titratable acidity (TA) and pH

The change in titratable acidity of fruit during storage in presented in (Figure 3.7 a, b). Results show an initial TA of 2.0 g/L and 0.7 g/L for 'Wonderful' and 'Bhagwa,' respectively. There was a decreased in TA as storage progressed with an insignificant margin of 0.1 to 0.4 g/ titratable acidity by the end of one-month storage across storage conditions. The decrease could be attributed to increased activity of citric acid glycosylase or conversion into sugars as reported by (Ratore *et al.* (2007) in mangoes. Hess-Pierce & Kader (2003) also reported a similar pattern of TA for a fruit stored in room air.

With specific regard to Xtend® film bags, it was observed that TA decreased to lower levels, and thereafter stabilised at 0.3 g/L for both cultivars at 5 °C. The results corroborate with those reported by Nanda *et al.* (2001) on the effect of wrapped films which also exhibited minimal weight loss due to the influence of films with different water vapour transmission rates (WVTR) properties.

'CA' storage had no influence in reducing TA (1.5 g/L and 0.6 g/L, respectively, at the end of one month (Figure 3.7). Additionally, the high retention of TA could be due to lowering biochemical activities by high enhanced CO_2 in as reported by Hess-Pierce & Kader (1984) and Kupper *et al.*, (1995). According to Kader *et al.* (1984) there a link between enzymatic breakdowns of nutrients in the Krebs cycle with high CO_2 and maintaining fruit quality. In our study, there was an increase in pH from the mean 3.3 to 4.2, respectively by the end of storage periods (Figure 3.8a, b). The atmosphere with 14% CO_2 had low influence on pH compared to other storage conditions, signifying a counteractive effect of the decreasing titratable acidity.

Total soluble solids / titratable acid ratio (TSS/TA)

The ratio of sugar to the acid is considered vital as it contributes to flavour development in most fruit species (Melgarejo *et al.*, 2000). In the study, the observed changes in TSS and TA during storage could have been responsible for the fluctuations in sugar /acid ratio. It was observed that TSS/TA ratio reduced by 50% in the first month possibly due to interactive effect of temperature and overall storage environment. The ranking order for the ratio revealed (CA2 > CA1 > XT5 > NA5) in which 'CA' -conditions retained higher ratio than fruits stored under room air at 5 °C, showing the least among storage conditions. According to Al-Said *et al.* (2009), the unique flavours of pomegranate is attributed to the superlative organic acid/sugar balance which the conditions under 'CA' has prospects to maintain including retaining fruit quality.

Colour

The colour of pomegranate during storage is shown in (Figure 3.9a, b) for cv. 'Wonderful' and (Figure 10a, b) for 'Bhagwa' pomegranate. It was observed that fruit stored in room air at 5 °C slightly decreased in redness chroma C* from 39 to 35.5 by the end of one month, after which remained stable (Figure 9a). The lightness L* remained within the 55 L* value. Fruits stored in XT5 bags showed a slight increase in colour chroma C*, and luminosity L* values within the ±2 margin from the initial 42 - 45, possibly due to low respiration rate as a result of increased gases as observed in Figure 3.1 earlier. The findings are also in agreement with findings reported by Hess-Pierce & Kader (1984) for non-climacteric fruits.

Fruits stored under 'CA' showed slight fluctuations (increase /decrease) in red colour expressed as Chroma C* value and L* values with time. CA2-sstorage with (5% O_2 + 14% CO_2) showed an increased in the first 30 days and stabilised thereafter for the rest of storage period. Results are similar to those reported in the literature (Elyatem & Kader, 1984; Kupper *et al.* 1995).

Texture

The mechanical properties expressed as puncture force required to cut the whole fruit varied significant between cultivars ($P<0.05$) (Table 1). The initial puncture force before storage, were 162 N and 155 N, for pomegranates 'Wonderful' and 'Bhagwa', respectively. The variation in mechanical force could be due to differences texture linked to genetic and agro-climatic conditions (Verma *et al.*, 2010), and also dissimilarities in peel thickness and maturity of fruits (Fawole & Opara, 2013). After storage, the coefficient variation (Table 3.1b) show (< 10%) variation in the samples tested.

After storage, fruits under room air at 5 and 20 °C showed an increase in firmness by 17 and 22%, respectively. In the case of fruits stored under 'CA' , the increases were proportionately lower by 50% compared to fruits stored in room air with 10 to 14%). Changes in mechanical properties could be linked to moisture loss resulting into lignification of the cell wall, and toughening as reported by (Kader, 2006). Similarly, transpiration is a major cause moisture loss, which impacts on the changes in cell layer structure thereby hardening of epidermal cells of the cuticle layer (Kader, 2006).

In the case of arils, the force before storage was 75 to 79 N which gradually changed by 1 to 6.4% for respective cultivars. In contrast to literature values, our results for arils were three-fold higher than those reported by Al-Said *et al.* (2009) for pomegranate cultivars grown in Oman in Arabian Gulf, with a firmness of range 24.6 to 27.0 N. The difference in texture can be attributed to genetic variation and geographic area of production. According to Fawole *et al.* (2013), textural

dynamics of aril tend to increase in bioyield force and elasticity as the fruit advances in maturity and during storage, a phenomenon that best describes changes of mechanical properties in our study.

CONCLUSIONS

The response in terms of physico-chemical properties (weight loss, decay, TSS, TA, Colour) varied depending on 'CA' , cultivars and duration. CA1 and CA2 storage system resulted in low weight loss (<10%), decay (<3%) which have both quality and economic implication. Overall CA2 with 5% O_2 + 14% CO_2 performed better than other treatments and extended shelf life for five months. In addition, the interaction effect of 'CA' , temperature and duration was possibly a cause of fluctuation trend of quality attributes observed during storage. Fruits stored in room air lasted only for two months due to high respiration in terms of oxygen consumption (RO_2) and carbon dioxide evolution (RCO_2) resulting into high physiological disorders. On the other hand, high respiration rate of fruits exhibited when taken out of 'CA' signify possible stress response and suggests the need for further studies on how best manage fruits once are taken out of 'CA' storage conditions. Xtend® film packaging minimised weight loss better than cold storage with room air. In addition, the Xtend® film bags at 5 ºC extended the postharvest life of pomegranate for three more months, though it requires further studies to optimise.

TABLE AND FIGURES

Table 3.1. Mechanical properties of whole pomegranate and arils before and after storage

	'Bhagwa'				'Wonderful'			
Property	Day 0	After storage	After storage	After storage	Day 0	After storage	After storage	After storage
	Day zero	RA5	CA1	CA2	Day 0	RA5	CA1	CA2
PR (N)	155.3 ± 0.8h	188.6 ± 0.2d	173.8 ± 2.6f	177.9 ± 1.8e	162.4 ± 1.3g	190.2 ± 0.1c	195.0 ± 0.5b	199.8 ± 1.9a
ARH (N)	75.9 ± 1.6b	84.4 ± 3.4a	76.0 ± 2.6b	80.8 ± 4.2ab	77.5 ± 2.1b	75.4 ± 1.3b	79.6 ± 1.3a	82.3 ± 2.1a

Column one and five represent values at day 0, while other columns represent values after storage for five months. PR= Puncture resistance of whole fruit (N), ARH=Aril hardness resistance (N) means (±SE) in each row followed by different letter (s) are significant different ($p<0.05$) based on Duncan multiple range test

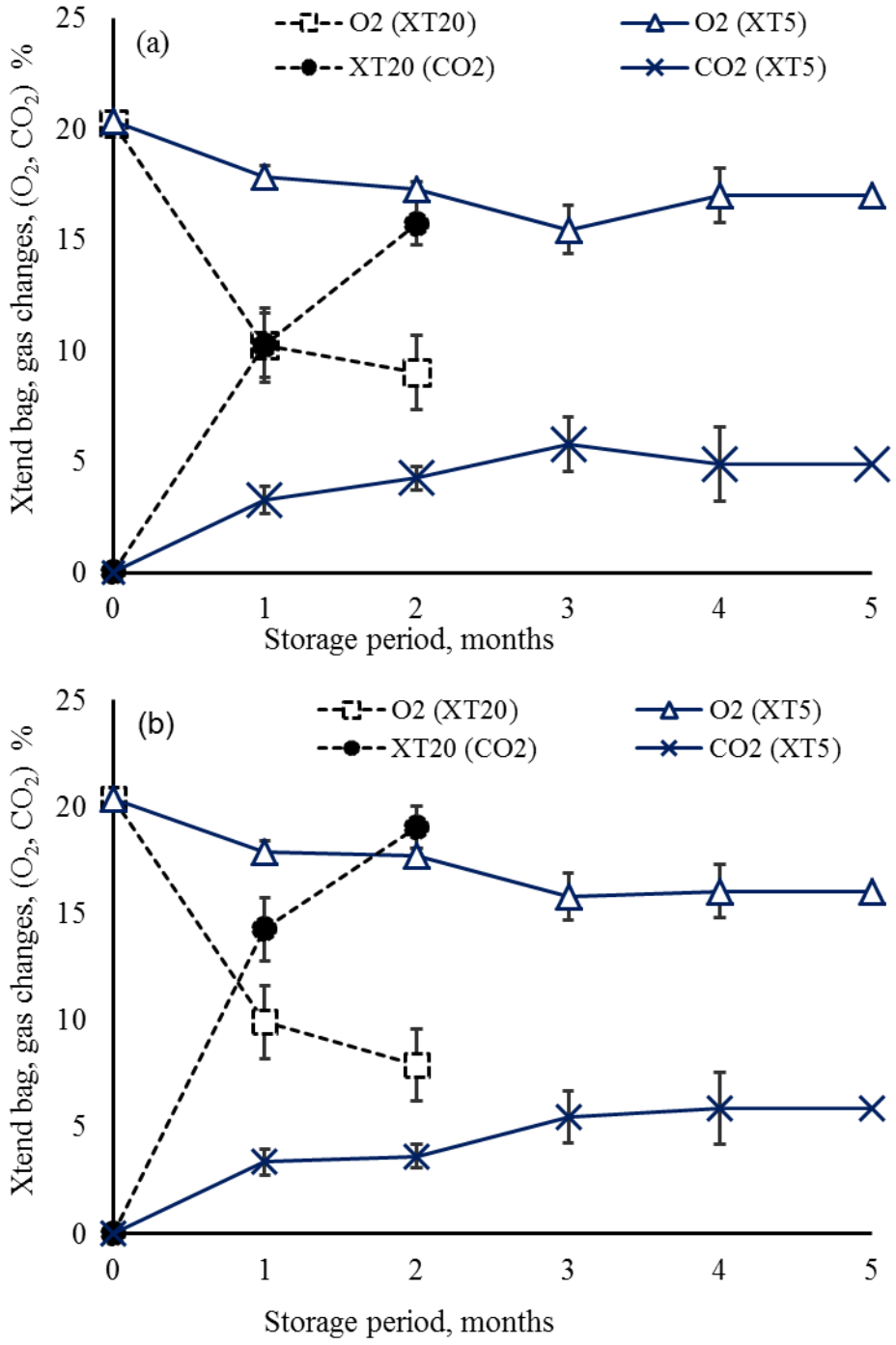

Figure 3.1. Changes in gas composition inside X-tend package (a) 'Wonderful' and (b) 'Bhagwa' XT5=XT20 = Xtend® film bag stored at 5 and 20 °C. Vertical error bars repesent standard error of the mean ± SE at $p<0.05$.

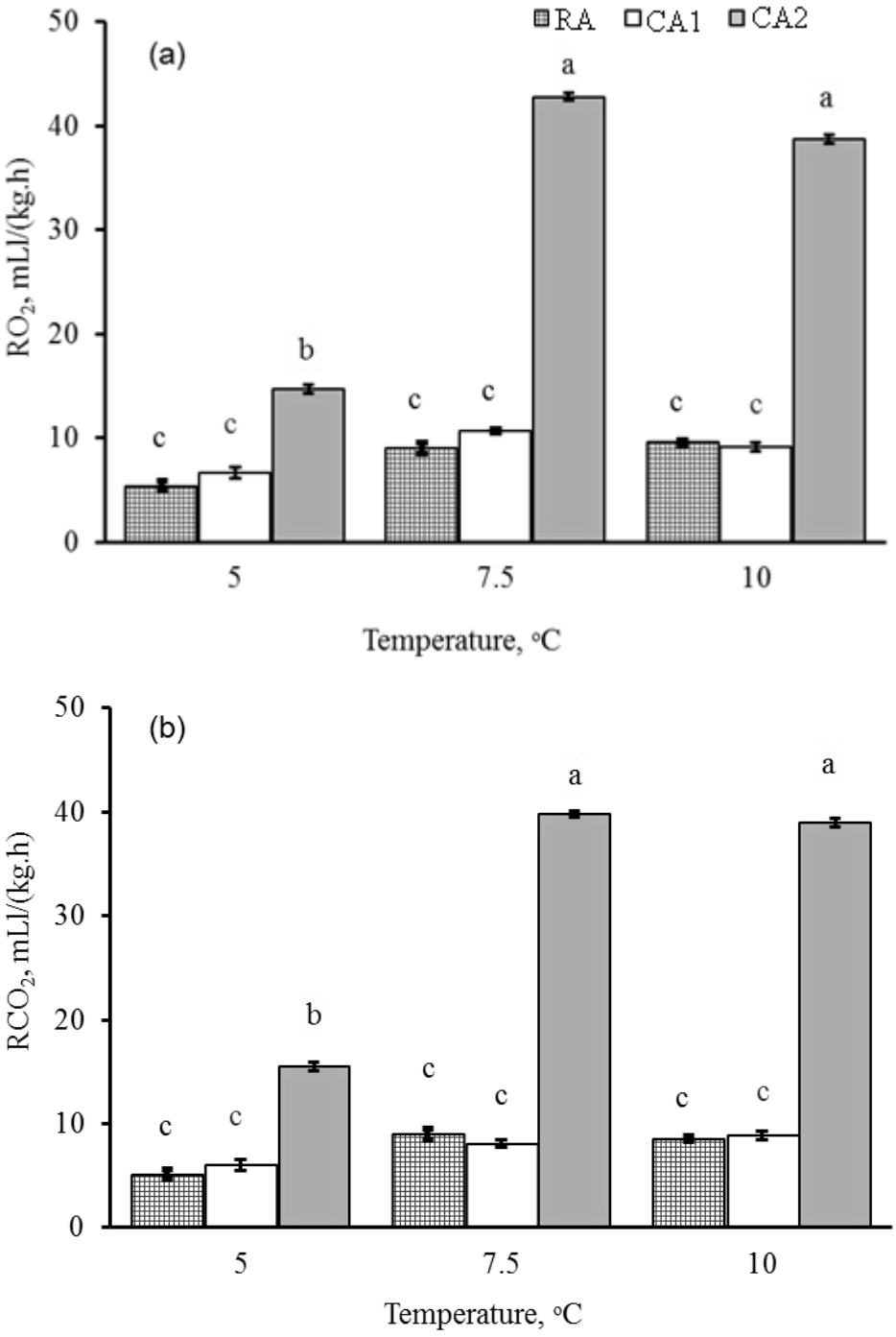

Figure 3.2. Respiration rate of pomegranate fruit (a) (RO_2 and (b) RCO_2) for (a) 'Wonderful'. Vertical error bars represent standard error of the mean. Different letters on bars indicate significant difference of mean ± SE at $p<0.05$, RA: Room air, 'CA' : 3% O_2 + 6% CO_2, CA2: 5% O_2 + 14% CO_2.

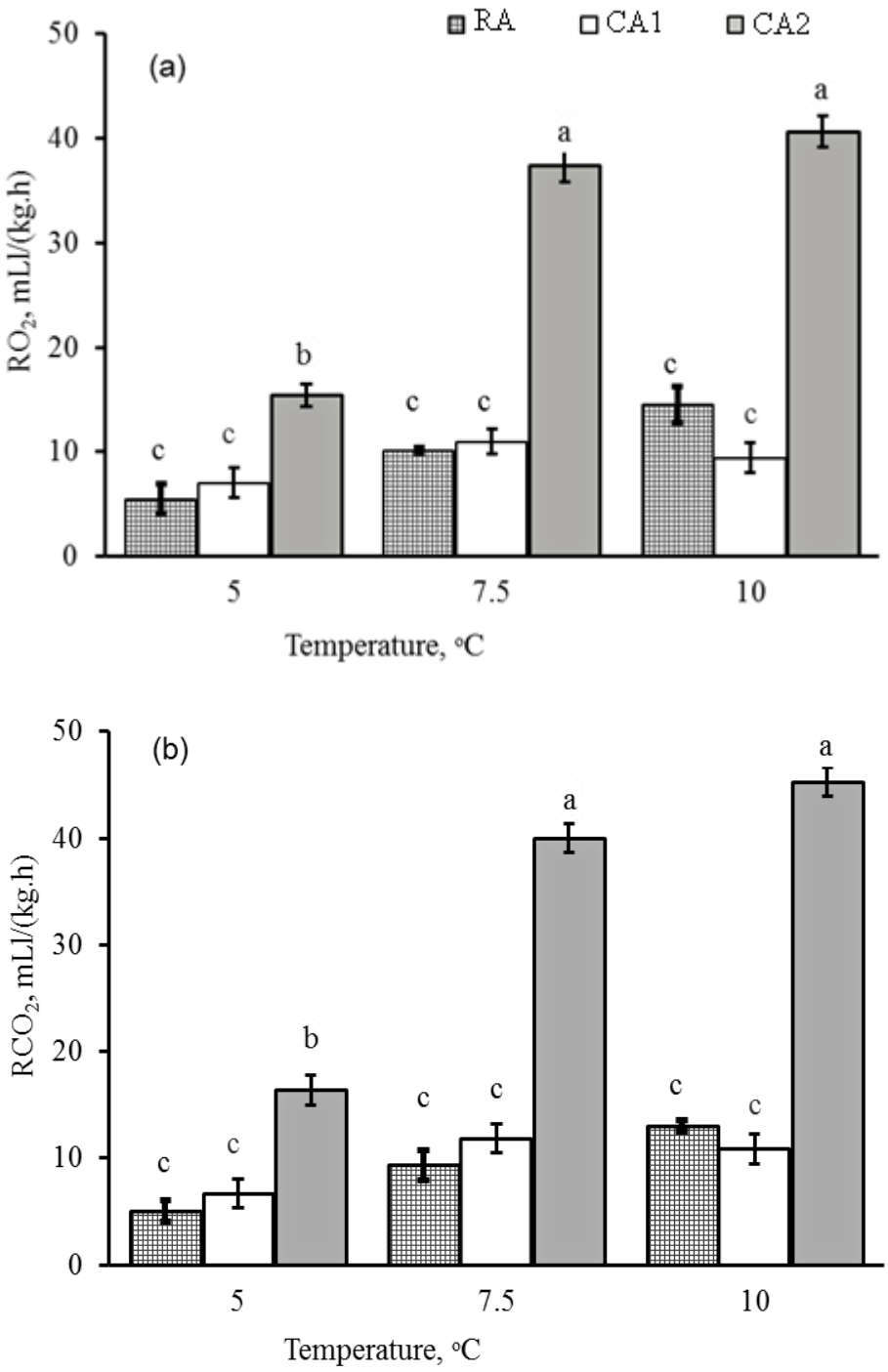

Figure 3.3. Respiration rate of pomegranate fruit (a) RO_2 and (b) RCO_2 for 'Bhagwa'. Vertical error bars represent standard error of the mean. Different letters on bars indicate significant difference of mean ± SE at $p<0.05$, RA: room air, 'CA' : 3% O_2 + 6 CO_2, CA2: 5% O_2 + 14% CO_2.

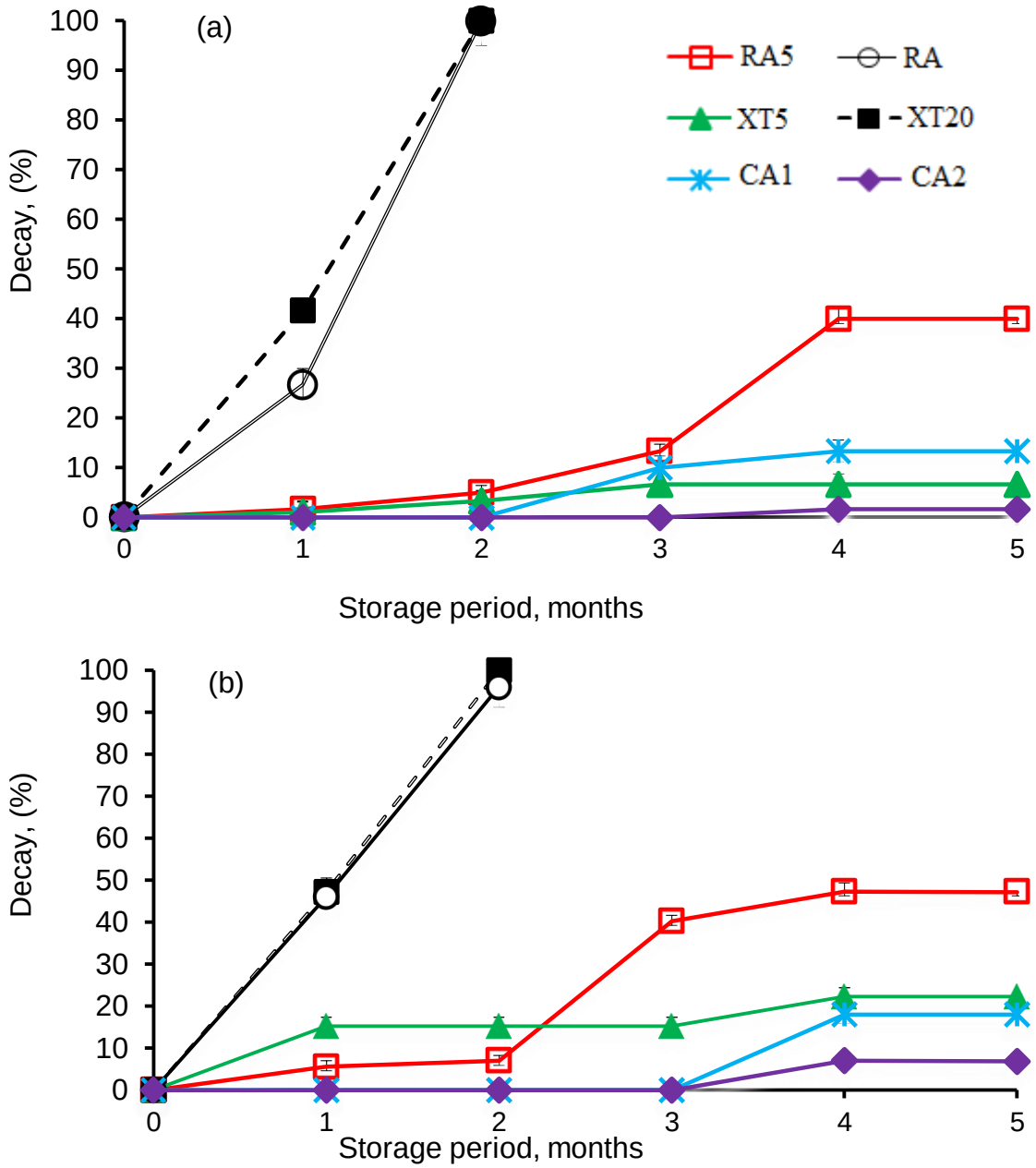

Figure 3.4. Physiological disorder of pomegranate fruit (a) 'Wonderful' and (b) 'Bhagwa' under. Different data points with error bars indicate mean ± SE at $p<0.05$, RA=room air at 5 and 20C°, XT=Xtend® film bag, CA1: 3% O_2 + CO_2, CA2: 5% O_2 + 14% CO_2.

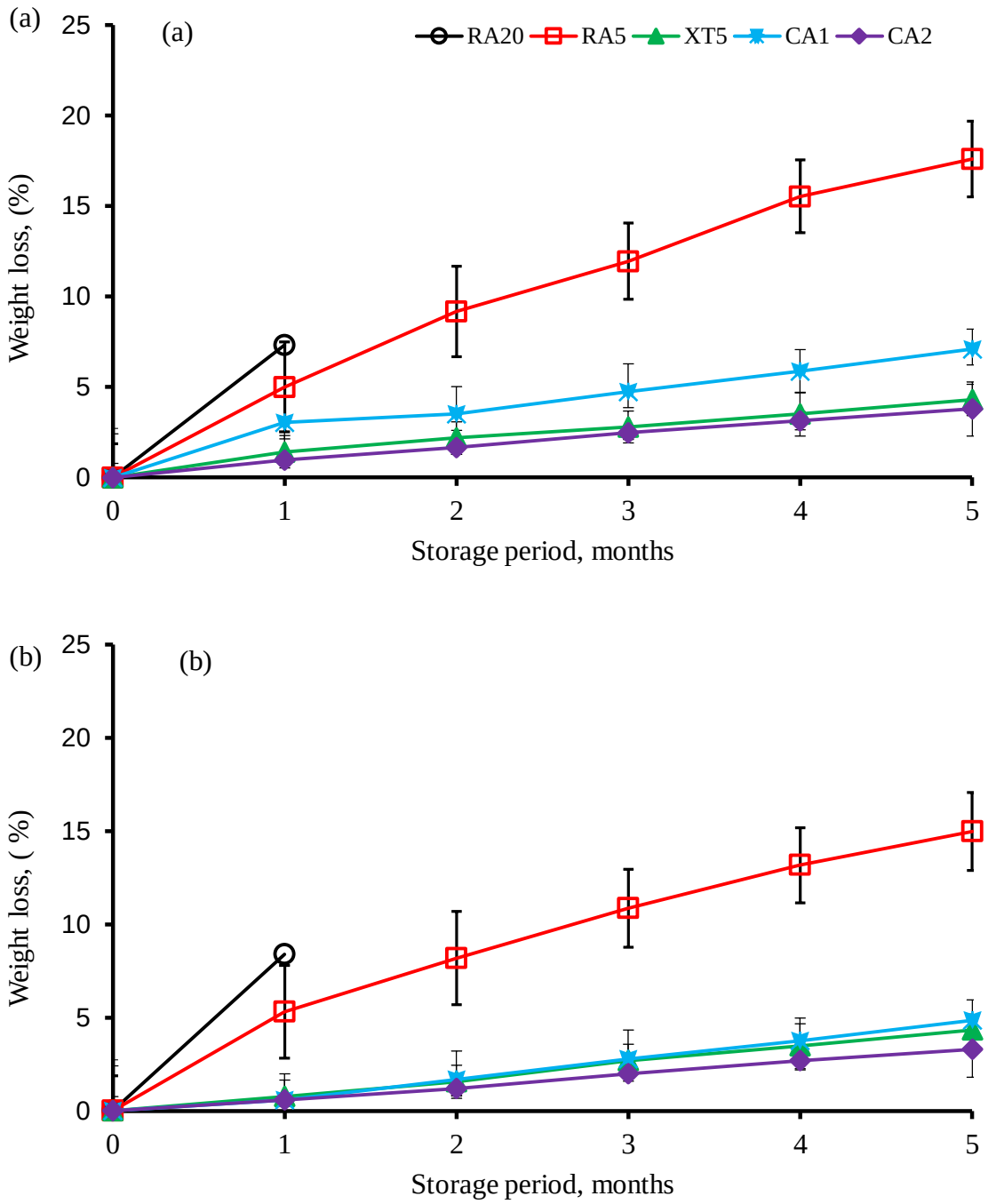

Figure 3.5. Weight loss of pomegranate fruit (a) 'Wonderful' and (b) 'Bhagwa' under different storage conditions (RA20 RA5 XT5 CA1 CA2), RA= Room air at 5 and 20 C°, XT=Xtend® film bag, CA1: 3% O_2 + CO_2, CA2: 5% O_2 + 14% CO_2. Different data points with error bars indicate mean + SE at $p<0.05$.

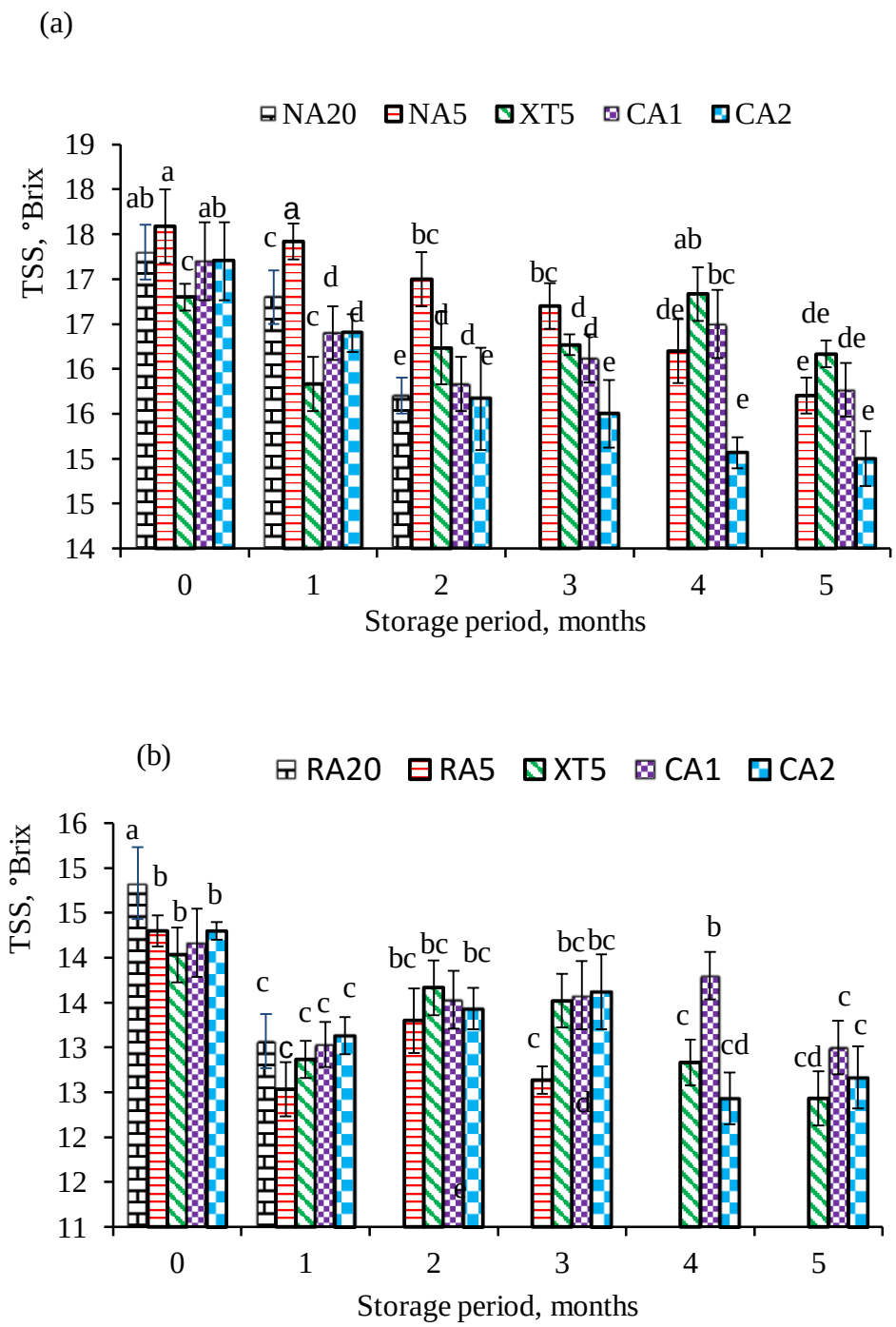

Figure 3.6. Total soluble solids (°Brix) of pomegranate fruit juice (a) 'Wonderful' and (b) 'Bhagwa' under different storage conditions, Vertical error bars represent standard error of the mean. Different letters on bars indicate significant difference of mean ± SE at $p<0.05$. RA= Rom air at 5 and 20 °C, XT= Xtend® film bag, CA1: 3% O_2 + CO_2, CA2: 5% O2 + 14% CO_2.

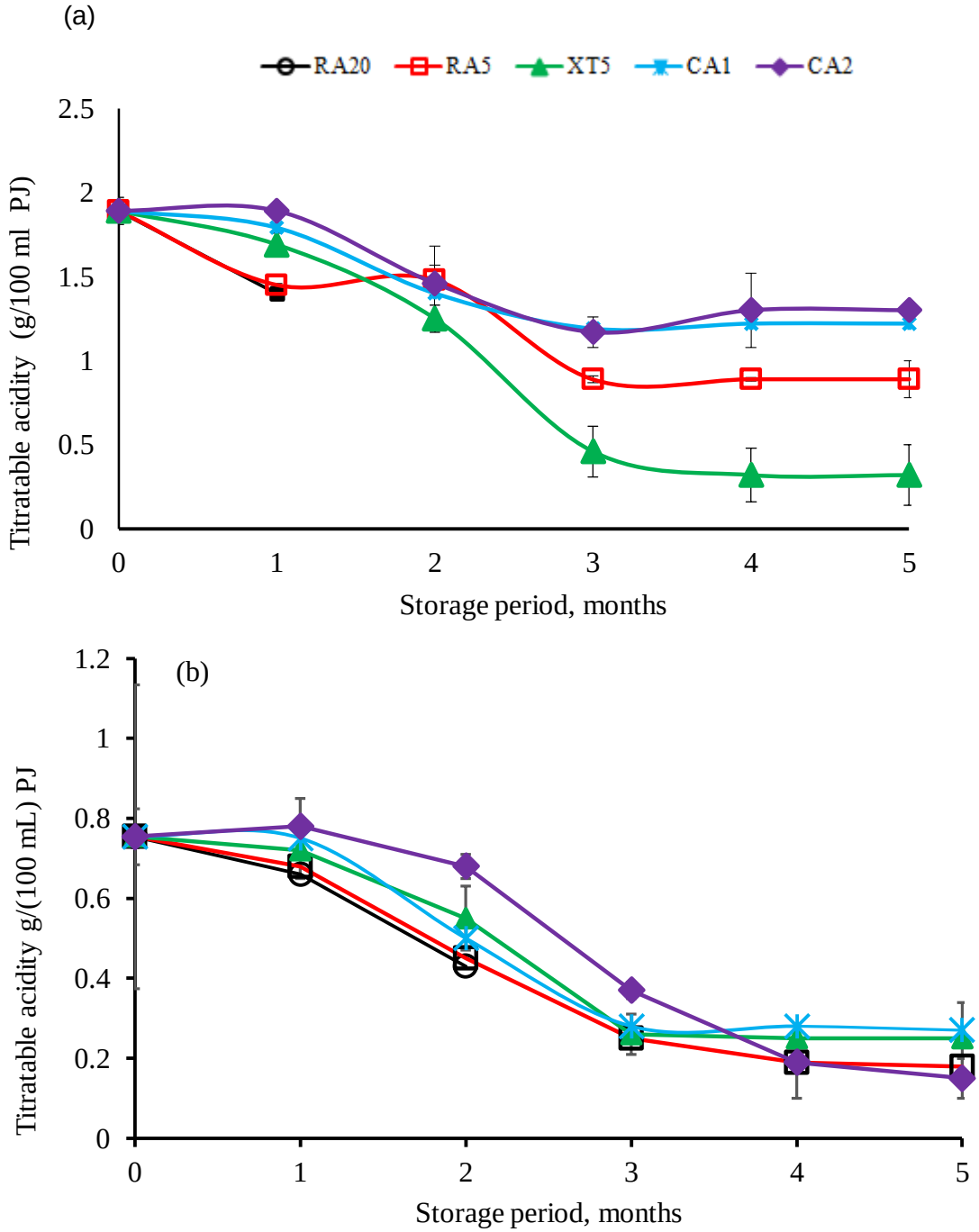

Figure 3.7. Titratable acidity of pomegranate juice (a) 'Wonderful' and (b) 'Bhagwa' under different storage conditions. Different data points with error bars indicate mean ± SE at $p<0.05$. RA= Room air at 5 and 20 C°, XT= Xtend® film bag, CA1: 3% O_2 + CO_2, CA2: 5% O_2 + 14% CO_2, PJ=Pomegranate juice.

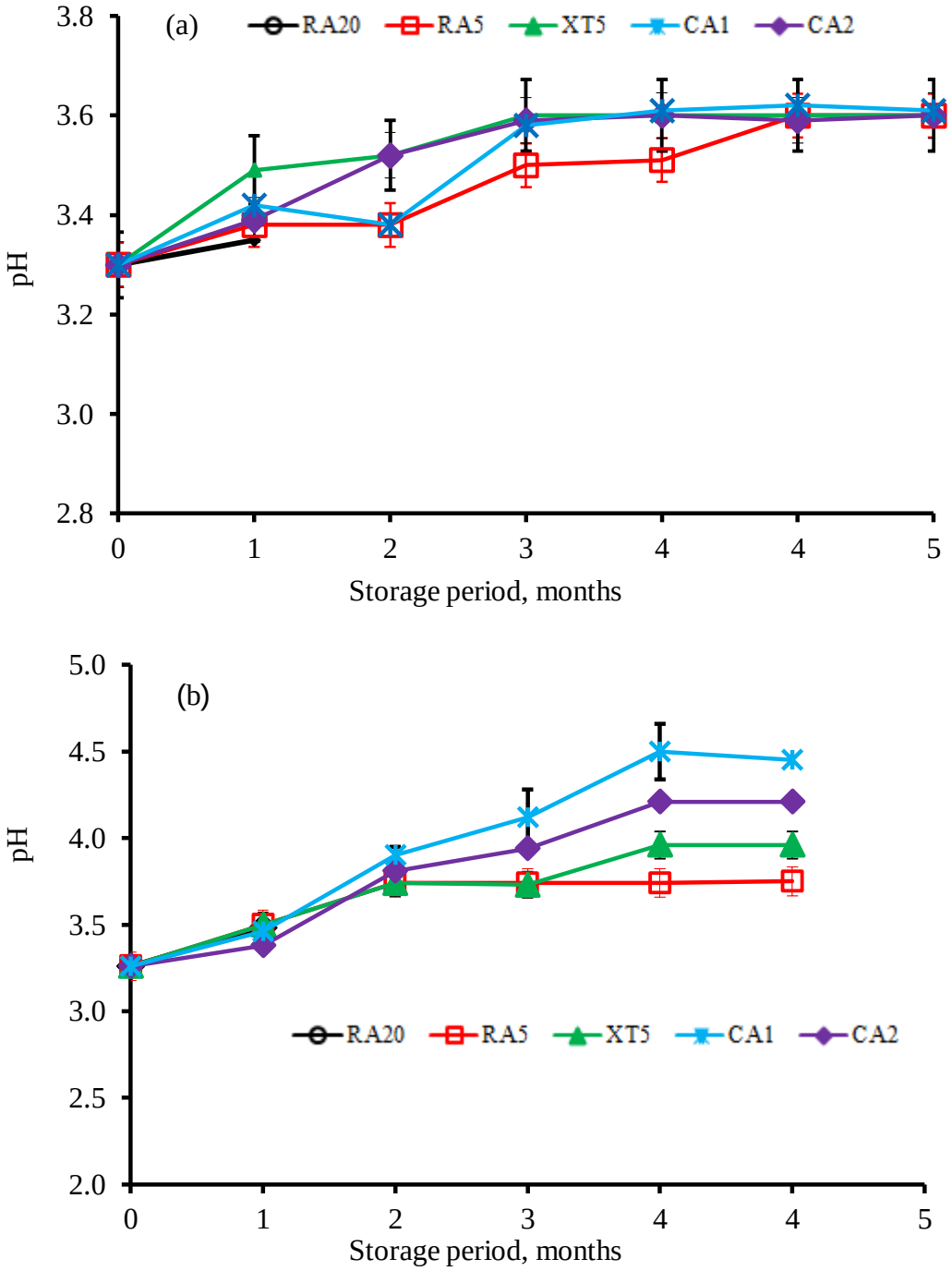

Figure 3.8. pH of pomegranate juice (a) 'Wonderful' and (b) 'Bhagwa' under different storage conditions. Different data points with error bars indicate mean + SE at $p<0.05$. RA= Room air at 5 and 20 °C, XT=Xtend® film bag, CA1: 3% O_2 + CO_2, CA2: 5% O_2 + 14% CO_2.

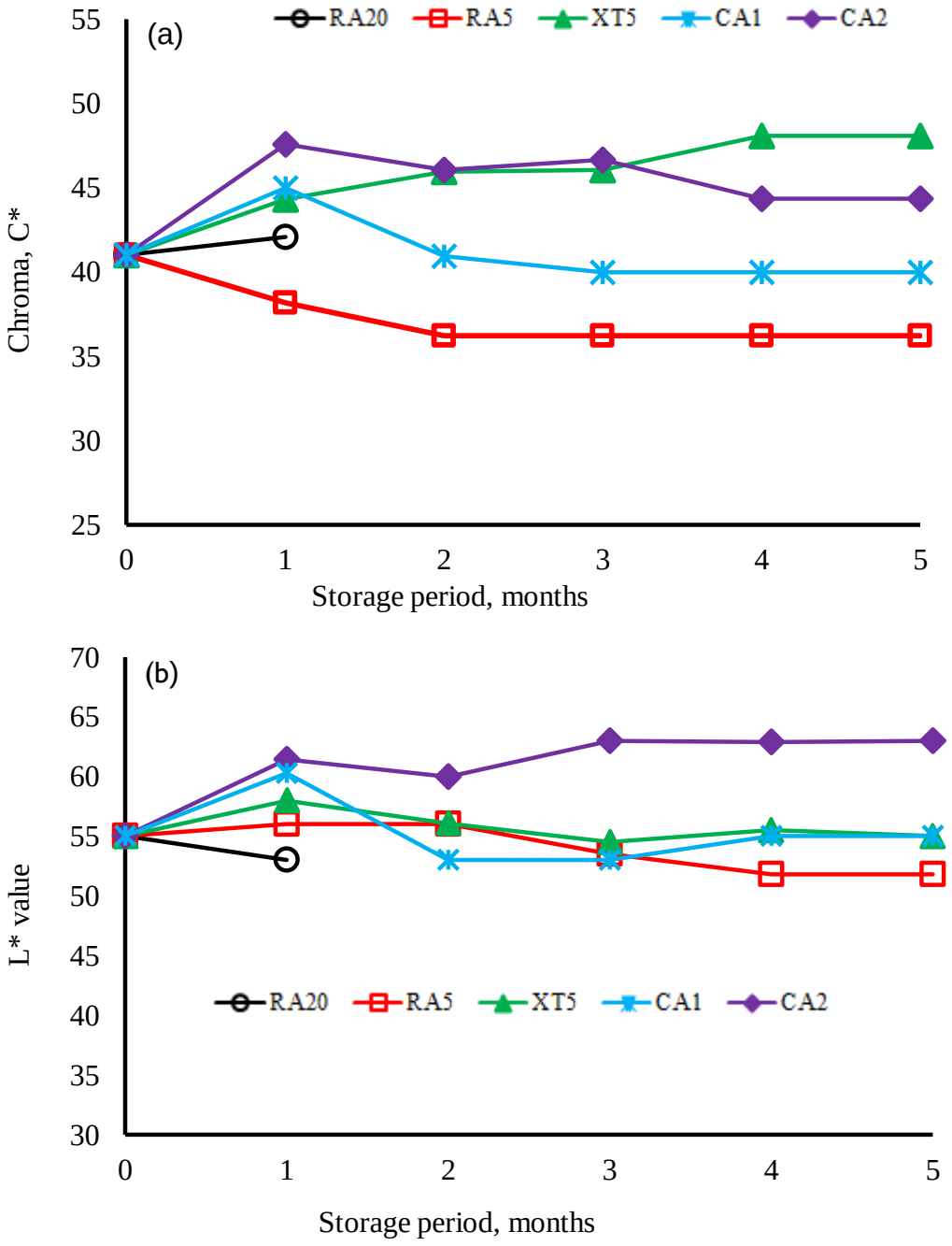

Figure 3.9. Colour changes (a) chroma C* and (b) L* values of pomegranate fruit skin (a) 'Wonderful' under different storage conditions . Different data points with error bars indicate mean + SE at $p<0.05$. RA= Room air at 5 and 20 C°, XT=Xtend® film bag, CA1: 3% O_2 + CO_2, CA2: 5% O_2 + 14% CO_2.

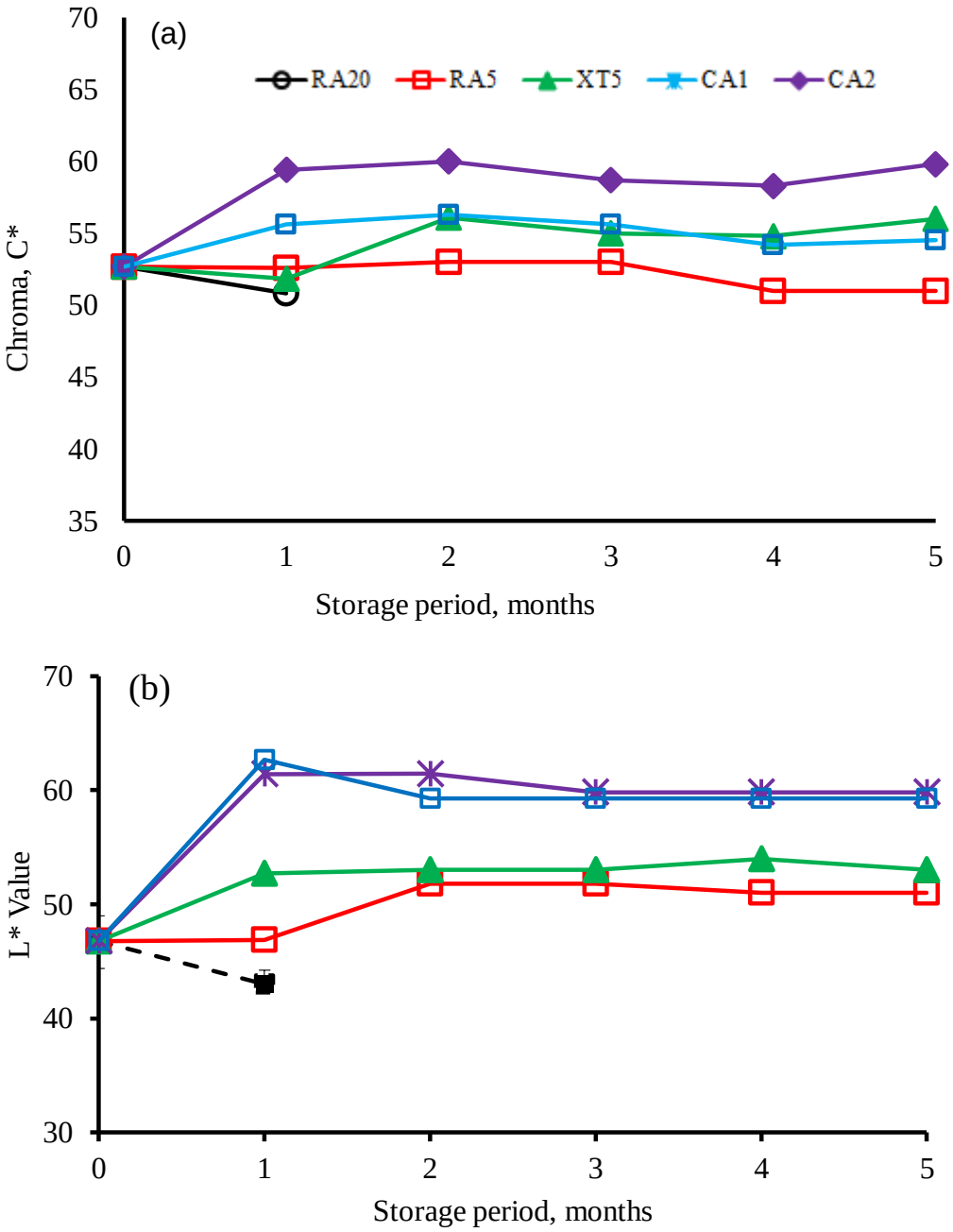

Figure 3.10. Colour changes (a) Chroma C* and (b) L* values of pomegranate fruit skin (b) 'Bhagwa' under different storage conditions Different data points with error bars indicate mean + SE at $p<0.05$. RA= Room air at 5 and 20 °C, XT=Xtend® film bag, CA1: 3% O_2 + CO_2, CA2: 5% O_2 + 14% CO_2.

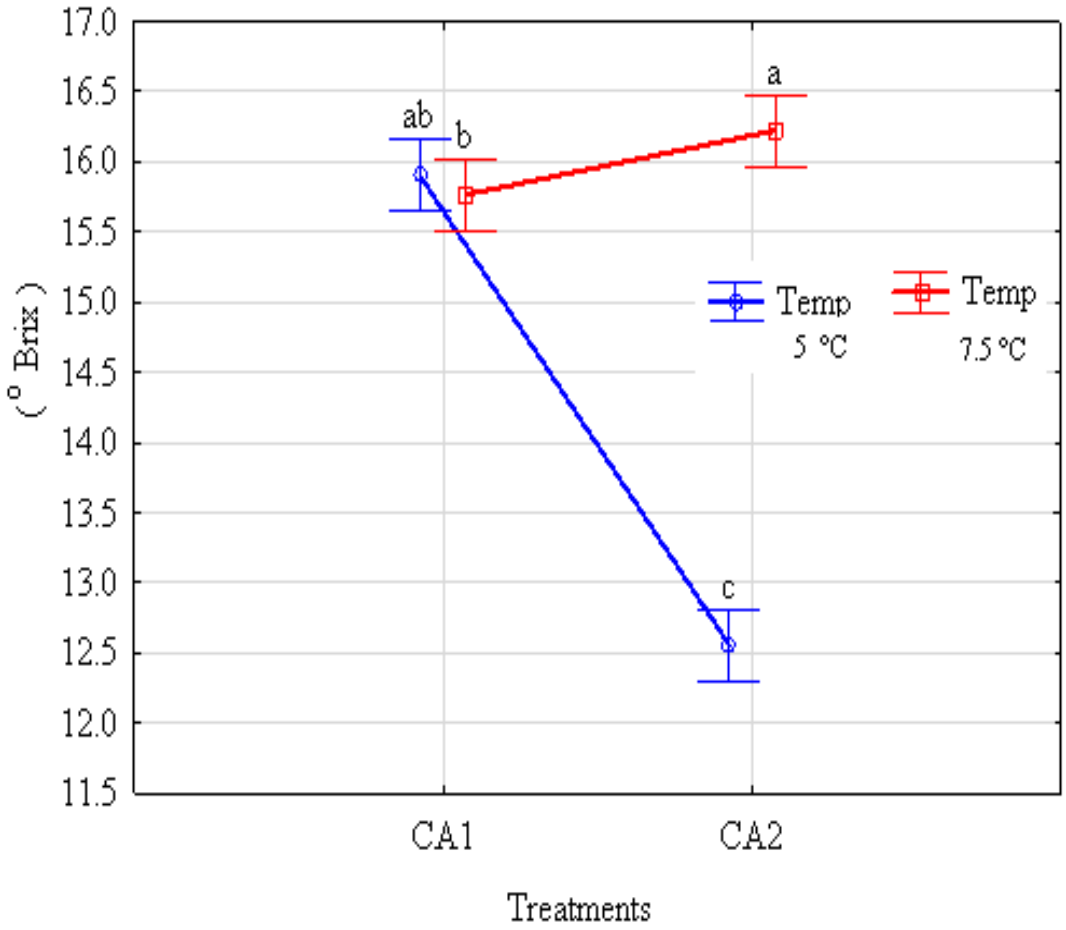

Figure 3.11. Effect of interaction between (CA1 or CA2) with temperature (5 or 7.5 °C) on TSS. Vertical bars denote $p<0.05$ confidence interval.

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CHAPTER 4

EFFECT OF 'CA' AND STORAGE TEMPERATURES ON ANTIOXIDANT PROPERTIES OF 'WONDERFUL' POMEGRANATE

Abstract

The effects of 'CA' and storage temperature on bioactive compounds (antioxidant activities and total phenolic content) during long storage were investigated. Two different 'CA' treatments (CA1; 3% O_2 + 6% CO_2 and CA2; 5% O_2 + 14% CO_2) and in room air (RA) (RA; 20% O_2 + 0.03% CO_2 at 20, 10, 7.5 and 5 °C conditions were investigated. Fruits were sampled on monthly basis for three months. At each stage, fruits were removed and stored for a further three days to simulate shelf life under room air. The evaluation of antioxidant activities (radical scavenging activity (RSA), ferric ion reducing antioxidant power (FRAP), diphenylpicrylhydrazyl (DPPH), total phenolic content (TPC) and ascorbic acid was done using recommended assays. Results showed that changes in antioxidant properties were temperature dependent and that 'CA' and duration played a significant role. The RSA decreased at 5 °C in both 'CA' conditions while at a higher storage temperature (7.5 °C) a gradual increase in RSA occurred, but not significant between treatments. The TPC decreased in all 'CA' treatments in the first month and remained stable throughout the storage period at both temperatures 5 and 7.5 °C. The FRAP increased slightly in the third months under CA2 at 5 °C, thereafter a slight decline in the level was observed. The interaction between treatments 'CA'), temperature and duration had insignificant influence on ascorbic acid at all treatment except in the last month of storage when a slight decrease was observed. These results form a basis for further investigation on a wide range of 'CA' conditions and storage temperature in order to optimise the storage with respect to antioxidant activities.

Introduction

The quality of fresh pomegranate is defined in terms of its overall nutritional values. *Punica granatum* L according to Seeram *et al.* (2008) is rich in antioxidant potency compared to a wide range of fruit juices. Pomegranate fruit is characterised by its high phenolic contents and antioxidant properties with the potential to preventing common diseases (atherosclerosis, chronic renal failure, and diabetes mellitus) and many others (Lansky & Newman, 2007, Jurenka, 2008; Faria & Cahau, 2011). However, not all quality characteristics can be preserved to the same extent during the postharvest process. Fawole & Opara (2013) investigated the effect of storage temperature on physiological

responses of pomegranate fruit. The findings showed that the physiological responses and quality of pomegranate were affected by storage condition. They reported that temperature (5 °C) contributed significantly ($p < 0.05$) to the reduction in the total phenolic content of fruit during prolonged storage beyond 8 weeks, although the levels of antioxidant activity in the fruit were not affected. More recently, Selcuk & Erkan (2015) evaluated the effects of modified atmosphere packaging (MAP) on phenolic compounds and antioxidant activity of 'Hicaznar' pomegranate during long-term storage. They also observed a fluctuation (showing an increase and/or a decrease) of total phenolic, total anthocyanin contents and antioxidant activity during the prolonged storage period. In other studies of MAP involving long-term storage of sweet 'Hicaznar' pomegranate, the total phenolic contents of pomegranates increased during storage and later declined to a steady-state atmosphere of (11-17% O_2 and 4– 5% CO_2) (Nurten & Erkan, 2016). It was evident that storage temperature and duration had a significant influence on antioxidant capacity, anthocyanin, phenolic compounds and overall quality. More specific, the antioxidant capacity, total phenolic, and anthocyanin slightly changed under 5-10 °C than at 0 °C. Arendse *et al.* (2014) reported similar observations. However, no scientific information is available on the effect of 'CA' on antioxidant activities on pomegranate the South African grown pomegranate. The objective of this study to evaluate the impact of 'CA' and storage temperature on antioxidant properties of cv. 'Wonderful' pomegranate.

Materials and methods

Fruit sample

Pomegranate fruit cv. 'Wonderful' grown in South Africa in the 2013/2014 farming season were bought from Wellington, Western Cape (33.63° 'S, 18.98° 'E). At the packhouse, the fruits were sorted to remove defective ones, thereafter; disinfection using 1% chlorinated water was performed, followed by air-drying and packing in the Xtend bags (StePac, Tefen, Israel). 50 boxes, each containing 12 - 18 fruits, depending on the size, 50 boxes were transported to the Postharvest Research Laboratory at Stellenbosch University. All boxes were stored at 5 °C, and 90% RH for 24 hours before commencing the storage experiments.

Storage conditions

Pomegranate fruits were stored in three different conditions (CA1; 3% O_2 + 6% CO_2), (CA2; 5% O_2 + 14% CO_2) and in room air with (20% O_2 + 0.03% CO_2) at temperatures (20, 7.5 and 5 °C). The fruits were stored for three months during which sampling was done on monthly basis followed by

holding for a further three more days under shelf life conditions at 20 °C before analysing the antioxidant properties.

Extraction process

The extraction of antioxidants was done in accordance with the method recommended by Fawole & Opara (2013). Approximately 1 mL of crude pomegranate juice was used, to which 29 mL of cold 50% aqueous methanol was added. The mixture was vortexed followed by sonication for 20 min in a cold-water bath and then centrifuged (Merk, Eppendorf AG, Germany) at 10 000 rpm for 5 min at 4 °C. The supernatant was collected and assayed for antioxidant capacity and phenolic components.

Methodology

Radical-scavenging activity (RSA)

The RSA of 1-diphenyl-2-picrylhydrazyl radicals (DPPH) was tested on bulked pomegranate juice (PJ) samples, each containing three fruit per treatment. Analysis of RSA was done in triplicate using the method described by Karioti *et al.* (2004) with some modifications recommended by Fawole *et al.* (2013). In the modification, 15 µL of the methanolic extract of PJ sample was diluted with 735-µL methanol in test tubes followed by the addition of 750 µL, 0.1 mM. Methanolic DPPH solution. The mixture was incubated in room air for 30 min in the dark. Thereafter, measurement of absorbance at 517 nm was done using a UV-vis spectrophotometer. The RSA of the extract was compared with ascorbic acid (1 000 µg/mL). A blank sample containing only methanol instead of the test sample or ascorbic acid was included and treated in a similar condition. After measurement of the intensity of colour 517 nm, RSA was calculated according to the formula described in equation (1).

$$\text{RSA}\ (\%) = [1 - (\text{A}\ /\text{B})\ \times 100] \qquad (1)$$

Where, **A** is the test is the absorbance of the reaction mixture containing the standard antioxidant or extract, and **B** is blank.

Total phenolic content (TPC)

The TPC was determined using a method described by Fawole *et al.* (2012). In brief, 450 mL of 50% methanol and 50 mL of PJ extract were mixed in a glass test tube. Folin-Ciocalteu reagent was then added to the juice sample and kept in the dark for incubation for 10 min. The TP concentrations are

determined by measuring absorbance spectrophotometrically at 725 nm. The results were expressed as mean ± SE (mg) of Gallic acid equivalents (mg GAE g^{-1} DM) of peel in triplicate samples.

Ferric ion reducing antioxidant power (FRAP)

The FRAP was measured calorimetrically as described by Bennie & Strain (1996) with a modification recommended by Fawole *et al.* (2012). The FRAP working solution containing mixtures of 50 mL of 300 mM acetate buffer, 5 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 5 mL of 20 mM ferric chloride all freshly prepared were incubated in a water bath at 37 °C prior to usage. In triplicates, 150 µL aqueous methanolic pomegranate juice extracts were added to 2850 µL of the FRAP working solution before incubation in the dark for 30 min. The reduction of the $Fe^{(3+)}$ -TPTZ complex to a coloured $Fe^{(2+)}$-TPTZ complex at low pH was monitored by measuring the absorbance at 593 nm. Trolox (100 – 1000 µM) was used for the calibration curve, and the results are expressed as Trolox (mM) equivalents per mL pomegranate juice (mM TE/ml).

Ascorbic acid (AA)

The AA of pomegranate juice was determined in triplicate using the colorimetric method described by Fawole *et al.* (2013). One mL of bulked PJ was diluted with 1% metaphosphoric acid (MPA), vortexed and sonicated for 5 minutes in cold water, then centrifuged at 10 000 rpm for 5 min at 4 °C. The samples were further diluted with 0.0025% 2, 6-dichlorophenolindophenol dye followed by incubation in a dark environment for 10 min. The amount of Ascorbic acid in PJ was finally measured using a spectrophotometer at 510 nm. The results of AA were obtained extrapolating using a standard curve of known concentration of L-ascorbic acid (Sigma) and results expressed as mean ± SE (mg) ascorbic acid per 100 mL of crude juice.

Statistical analysis

Results were subjected to analysis of variance (ANOVA) using Statistica software (Statistica 13.0, StatSoft Inc., and Tulsa, OK, USA) and final mean results reported with standard error (± S.E) according to Duncan's multiple range tests.

RESULTS AND DISCUSSION

Radical scavenging activity (RSA)

The results of the study showed that there were significant (P<0.05) differences in measured RSA (Figure 4 a & b). The storage temperature and duration had a significant (p<0.05) influence in reduction of RSA by 27%, which stabilised until the second month in both 'CA' treatments at 5 °C. Thereafter, RSA increased by 30% to a level similar to the baseline values. However, the trend was different at a 7.5 ºC, where the RSA showed a progressive increase in all treatments with no significant (p<0.05) difference between the two treatments (CA1 & CA2), the response of the bioactive compounds to storage temperature and duration has been reported by several scholars (Fawole & Opara 2013; Selcuk & Erkan 2016). The researchers observed similar changes of fluctuating with an increase and/decrease of bioactive compounds during storage. For example, under MAP with 4.4–50% CO_2. Miguel *et al.* (2004) and Selcuk & Erkan (2015) reported similar findings showing the increase and/or decrease during storage. It can be concluded that changes bioactive compounds are mostly influenced by the interaction between 'CA' factors and storage temperature and duration. Results for RSA under both 'CA' storage at 7.5 C were progressively remarkable. .

Total phenolic content (TPC)

The TPC results are presented in (Figure 2 a & b). The TPC under 'CA' the storage showed a significant (p<0.05) decrease in all treatment in the first one months, followed by a stability at 400 GAE/1000 at 5 °C. At storage temperature (7.5 °C), a similar trend was observed in the first month, thereafter fluctuated with a significant (p<0.05) difference between the two 'CA' treatments. These results corroborate with findings reported by Selcuk *et al.* (2016). These authors attributed a fluctuation (increase and/or decrease) to oxidative stress which induces the synthesis of phenolic compounds. Similarly, Kader (2002) showed evidence that postharvest handling have a significant (p<0.05) effect the antioxidant capacity and phytonutrient levels in fruits depending on the cultivar, temperature and storage duration.

The ferric reducing power (FRAP)

Results of FRAP are presented on (Figure 4.3 a & b). It was observed that FRAP was temperature dependent showing a minimal fluctuation (increase and/or decrease) in antioxidant capacity of PJ under 'CA' at 5 °C. Selcuk & Erkan (2016) reported similar results for pomegranate 'Beynari' for

Turkish grown pomegranate stored under MAP with high levels of CO_2 in the atmosphere. There was a slight decrease in FRAP for pomegranate stored under CA1, while the level appeared to increase under CA2 under the same 7.5 C storage temperature, with storage period. The results are in agreement with those reported by Selcuk & Erkan (2015). They reported changes in bioactive compounds (phenolic compounds and antioxidant) activities of 'Hicaznar' stored under MAP.

Ascorbic acid (AA)

Ascorbic acid as a phytochemical compound found in citrus fruits plays many important functions in the human body. It functions as an antioxidant compound along with its traditional role of growth and repair of tissues in all parts of the body (Saxena *et al.*, 2009). In this study, the baseline value of ascorbic acid in cv. 'Wonderful' pomegranate was 15.32 mg/100 mL of PJ (Figure 4a & b). The ascorbic acid remained constant during storage under 'CA' at 5 °C up to the 2 months, thereafter, the level decreased slightly by 0.13% in all treatments. A similar trend was observed for CA1 at 7.5 °C, unlike under CA2 with higher (14% CO_2) conditions where the levels fluctuated within the margin of ±0.01 mg. These results seem to indicate the sensitivity of ascorbic acid to both temperature and CO_2 levels. At low storage temperature (5 °C) and low (6% CO_2), the performance was outstanding compared to higher temperature and higher CO_2 levels under CA2. Previous studies on storage of pomegranate under 'CA' revealed that low metabolic alteration occurs when gas composition and temperatures are optimal. The results are in agreement with Kupper *et al.* (1995) who reported that low O_2 concentration and/or slightly enhanced CO_2 have little effect on the ascorbic acid levels during storage. Further studies have shown that most commodities stored at 1-4% O_2 and enhanced CO_2 has little or no effect on AA degradation during storage (Lee & Kader, 2000). Based on the above facts, it is evident that the results of this study are consistent with the findings reported in these studies, that CA1 at 5 °C could be optimal for this specific quality attribute.

CONCLUSIONS

The data presented in this study indicate that 'CA' and storage temperatures had a significant ($p < 0.05$) effect on the bioactive compounds. More specific, the interaction effect of 'CA' and temperature influenced changes in bioactive compounds. A steady improvement of RSA at 7.5 °C and, the stability of FRAP combined with insignificant ($p<0.05$) changes in ascorbic acids at 5 °C are positive contribution of 'CA' storage conditions. In comparing the 'CA' with room air storage conditions, the results suggest that the reduction and/or slight increase of some antioxidant properties caused by the oxidative stress could be optimised using a goal-oriented approach. This observation is

the first to be reported as such, forms a basis for further research on ‘CA’ -stored pomegranate in this area.

FIGURES

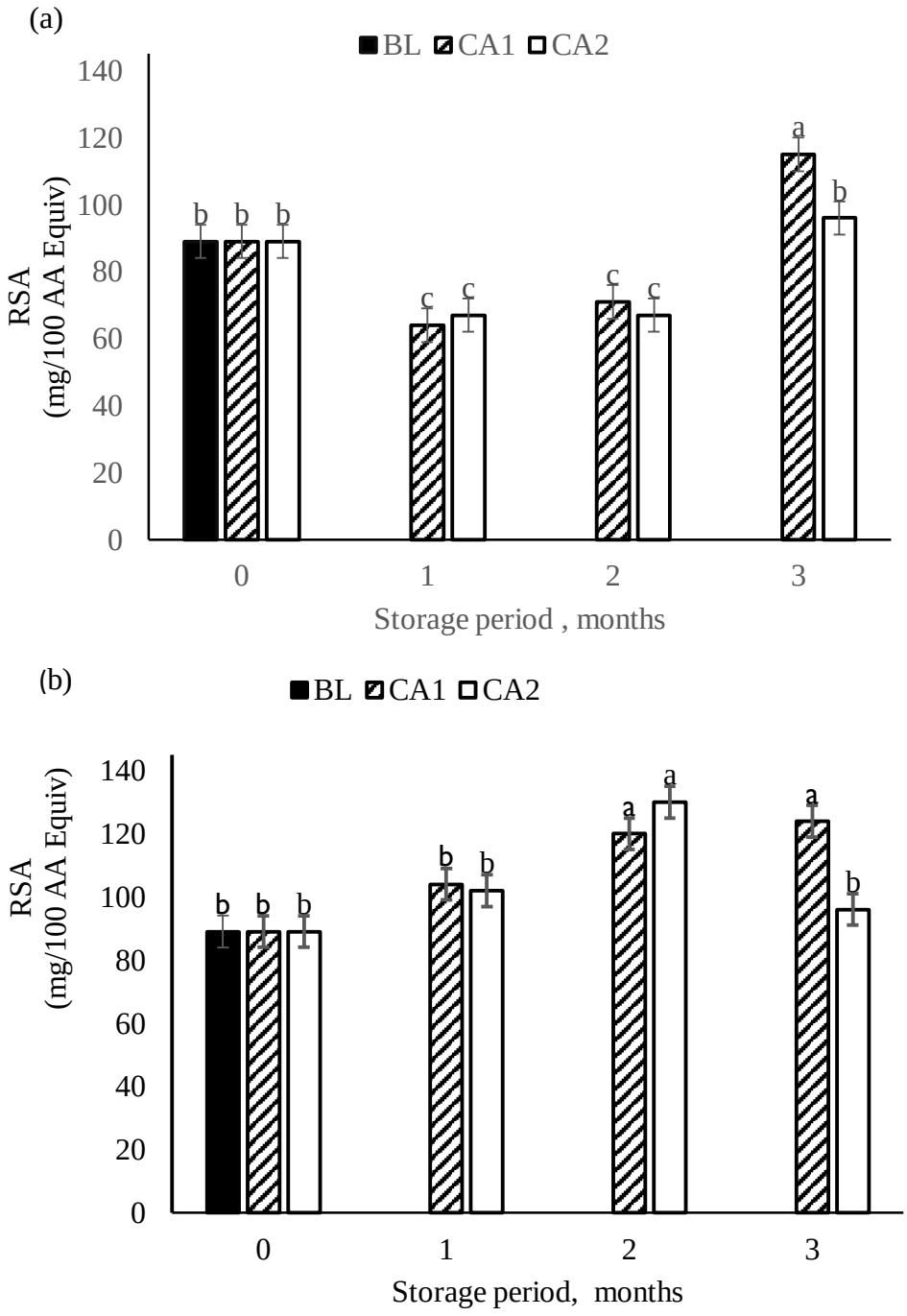

Figure 4.1. Radical scavenging activity (RSA) expressed as (ascorbic acid mg/100 mL) of pomegranate fruit juice stored in BL=Base line, CA1= 3% O_2 + 6% CO_2; CA2 = 5% O_2 + 14% CO_2, at (a) =5 °C and (b) = 7.5 °C. Different letter(s) indicate significant difference ($p < 0.05$) according to Duncan multiple range tests

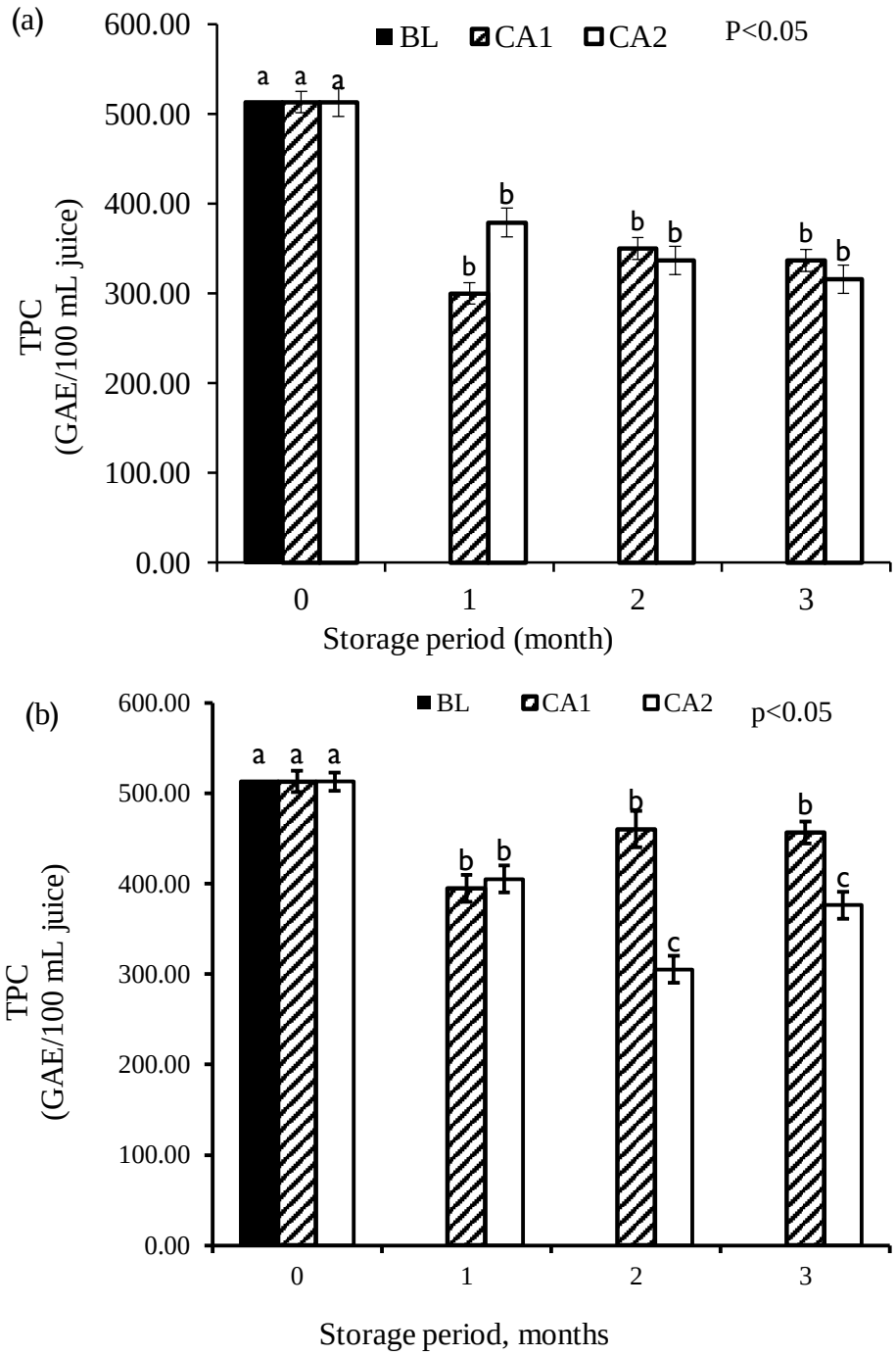

Figure 4.2. Changes in total phenolic concentration (TPC) (Gallic acid equivalent) of pomegranate fruit juice stored in BL=Base line, CA1= 3% O_2 + 6% CO_2 and CA2 = 5% O_2 + 14% CO_2 at (a) 5 and (b) 7.5 °C, respectively. Different letter (s) indicate significant difference ($p < 0.05$) according to Duncan's multiple range test

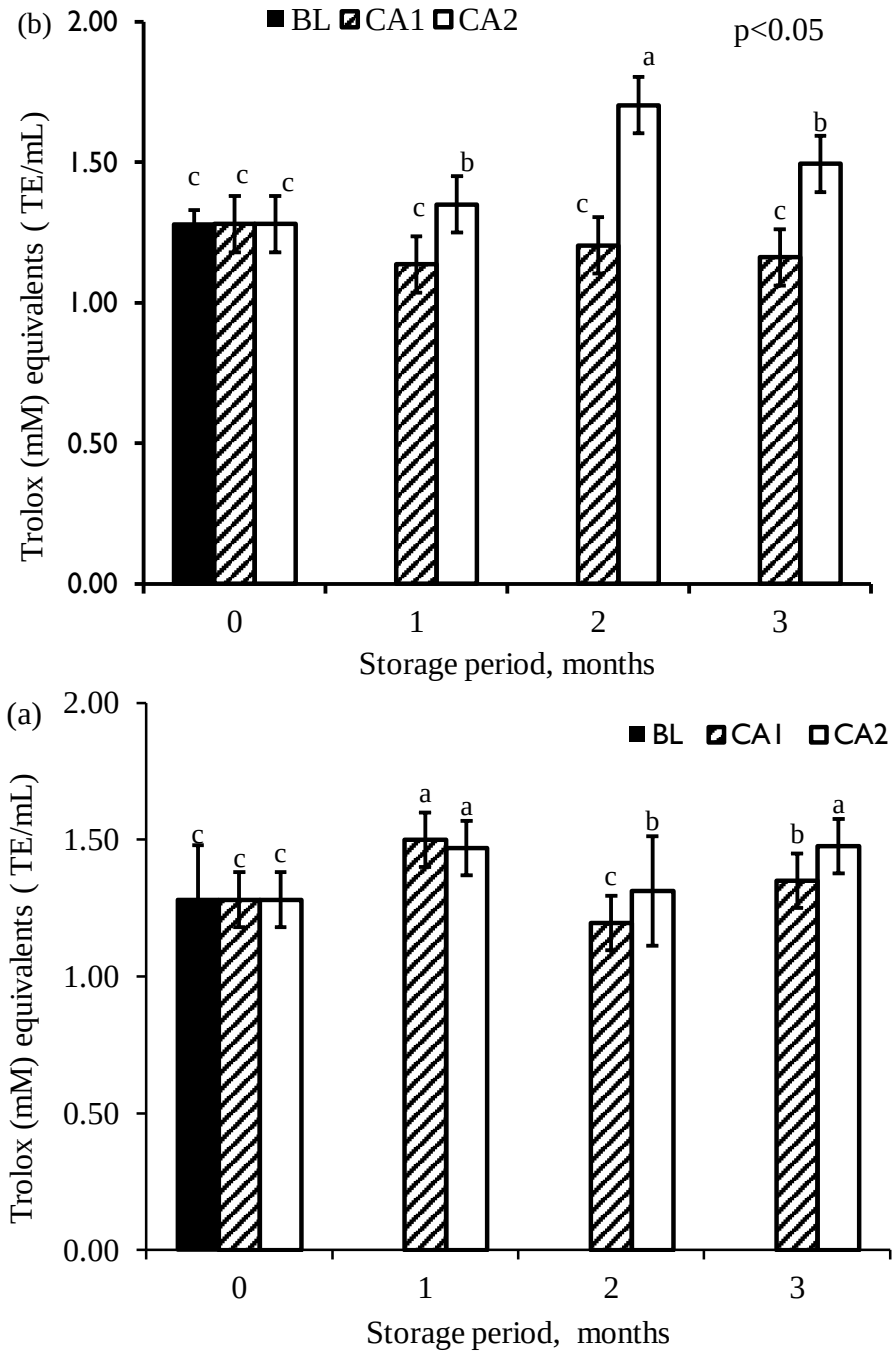

Figure 4.3. Changes in total antioxidant activity (Trolox mM) equivalents (TE/mL) of pomegranate fruit juice stored in BL=Base line, CA1= 3% O_2 + 6% CO_2 and CA2 = 5% O_2 + 14% CO_2, respectively, at (a) 5 °C and (b) 7.5 °C. Different letter (s) indicate significant difference ($p<0.05$) according to Duncan's multiple range test.

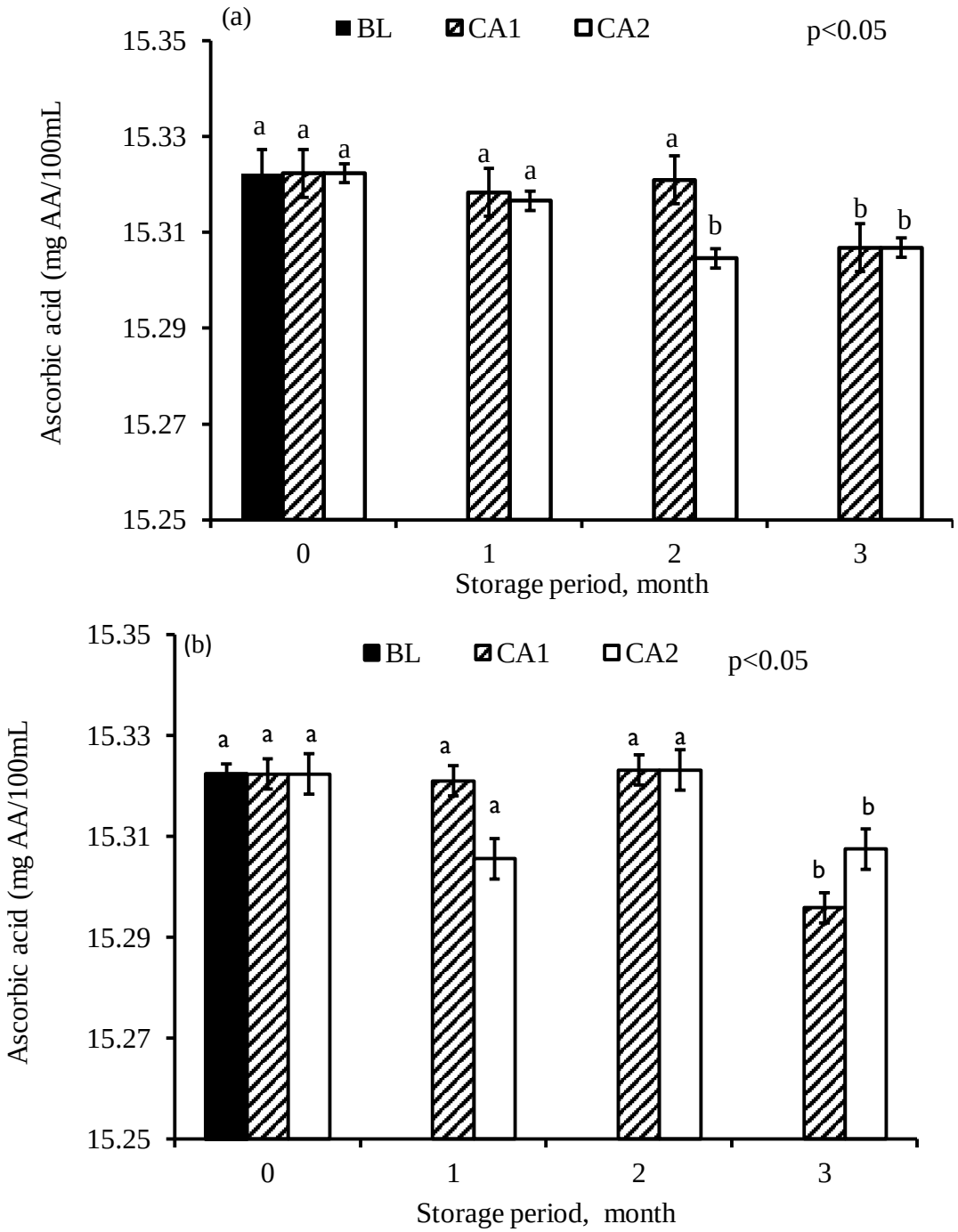

Figure 4.4. Changes in vitamin C (mg ascorbic acid equivalent/100 ml) of pomegranate juice stored in BL=Base line, CA1= 3% O_2 + 6% CO_2 and CA2 = 5% O_2 + 14% CO_2, respectively at (a) 5 °C and (b) 7.5 °C. Different letter (s) indicate significant ($p < 0.05$) differences according to Duncan multiple range test.

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CHAPTER 5.1

A KINETIC MODEL OF TRANSPIRATION IN CONTROLLED ATMOSPHERE STORAGE OF cv. 'WONDERFUL' POMEGRANATE

Abstract

Transpiration is an important physiological process that affects the physiological and other quality characteristics of pomegranate. A transpiration model based on heat and mass balances between whole pomegranate fruit and controlled atmosphere was developed and tested experimentally to predict moisture loss of fresh pomegranate in a controlled atmosphere at 5 ^{o}C and 96% relative humidity. Respiration heat (Qr) was obtained from measuring the respiration rate of O_2 consumption and CO_2 evolution. The convective heat transfer was calculated from an empirical formula for natural convection, in which the pomegranate temperature was taken as wet bulb temperature. The transpiration rate obtained by the developed model was 0.393 g/kg.h whereas the TR obtained experimentally (TR = 0.37 and 0.59 g/kg, h) for CA1 and CA2, respectively. By way contrast, the model prediction accuracy of R^2 = 0.97 goodness of fit to predict the weight loss over time was obtained. In addition, the kinetic model is simple, accurate and reliable. Although the respiratory heat (Qr) for CA1 and CA2 varied, the differences in TR was not significant.

Introduction

Transpiration is the process by which fresh fruits and vegetables lose moisture. This process includes the transport of moisture through the skin of the commodity, the evaporation of this moisture from the commodity surface and the convective mass transport of the moisture to the surroundings. Experimental studies have shown that any loss of fresh weight result in an economic loss if the commodity is sold by weight (Nunes & Edmond, 2007; Mahajan *et al.*, 2008). Empirical studies have shown that transpiration has a significant ($p<0.05$) impact on compositional changes in fruits (Kader et al., 2006). The changes in fruit turgidity, discolouration, flavour and overall nutritional quality when the weight loss reaches within 3-10% threshold depend on the particular produce (Kader *et al.*, 1984; Mahajan *et al.*, 2008). The driving factors that influence transpiration are two folds (Sastry & Buffington, 1982). They are categorised as intrinsic factors (surface-to-volume ratio, surface injuries, morphological) and extrinsic factors (temperature, RH, air movement, and atmospheric pressure (Sastry & Buffington, 1982; Chourasia *et al.*, 2005). In order to minimise losses due to transpiration, and thereby increase both market quality and shelf life, pomegranate fruits must be stored at a low

temperature, high humidity environment. Once harvested, metabolic activity in fresh fruits and vegetables continues being driven by the respiration process (Toledo *et al.,* 1969; Kader, 1989; Song *et al.,* 2002). The respiration involves the oxidation of sugars to produce carbon dioxide, water and heat. The resultant heat (Qr) influenced the escape of moisture just below the surface of a fruit (Sastry & Buffington, 1982). A properly regulated level of O_2 and CO_2 in the atmosphere can effectively reduce the rate of respiration and loss of moisture. Controlled atmosphere is once such a technology that has been studied and evidence show that the respiration rate in pomegranate can be reduced (Hess-Pierce and Kader, 2003). Kang & Lee (1998) used a similar principle of a kinetic model based on heat and mass transfer under 'CA' with (3% O_2 + 3% CO_2) to determine the transpiration rate of apples. Previously, most of the empirical studies reported models based on vapour pressure deficit (VPD) (Leonardi *et al.,* 2000; Mahajan *et al.,* 2008; Caleb *et al.,* 2013; Aindongo *et al.* 2014). To our knowledge, the non-invasive technique to develop a model for predicting the transpiration rate of pomegranate stored under 'CA' has not been reported. Therefore, the objective of this study was to develop a kinetic model to predict transpiration rate for 'CA' - stored pomegranate grown in South Africa.

Materials and methods

Pomegranate (*Punica granatum* L.) fruit cv. 'Wonderful', harvested manually during commercial harvest period were obtained from Robertson valley farm, Western Cape (33°480 S, 19°530 E), South Africa. Fruits were transported to the postharvest laboratory at Stellenbosch and stored at 5 °C before the start of the experiment. The individual fruits were removed from Xtend® film packaging , placed inside the glass jars of about 5L, and equilibrated at 5 °C for 24 hours prior to the experiment.

Experimental methods

Measurement of respiration rate

Respiration of pomegranate was measured by a closed system following a three day of equilibration at the desired temperature as described by Caleb *et al.* (2012). The air was flushed out using N_2, gas to remove excess O_2, until a required level of O_2 and CO_2, was achieved. Petroleum jelly was incorporated into the gap between the lid and jars to ensure no leakage of gas from the glass jar. The gases (O_2 and CO_2) were monitored hourly over a five-hour period using portable gas analyser (Checkmate 3, PBI Dansensor, Ringsted, Denmark). Respiration rates (RR) of O_2 consumption and CO_2 evolution were calculated from the linear regression curves. Slopes of regression lines were

multiplied by the free volume and then divided by sample weight to obtain respiration rates in mol/kg. h. Equations 1 and 2 refer to RO_2 and RCO_2 calculations.

$$R_{O_2} = R^i_{O_2} - \frac{R_{O_2}}{V_f} \times W \times (t - t_i) \times 100 \quad (1)$$

$$R_{CO_2} = R^i_{CO_2} + \frac{R_{CO_2}}{V_f} \times W \times (t - t_i) \times 100 \quad (2)$$

Where, $R^i_{O_2}$, $R^i_{CO_2}$, R_{O_2}, R_{co2} are, respectively, the O_2 and CO_2 concentrations in volumetric fraction in the gas mixture at the initial time t (hour) (or time zero) and at time t_i (hour). RO_2 and RCO_2 are the respiration rates (O_2 or CO_2/ mL/ (kg. h) and W is the weight of the pomegranate fruit (kg) and V_f is the free volume inside the glass jar (mL).

Model development

The transpiration model based on heat and mass transfer relation to the physiological behaviour pomegranate was developed based on the following assumptions (Song *et al.* 2002)

Assumptions

1. The fresh produce and the surrounding temperature are the same.
2. The headspace gas composition between the produce and the headspace is assumed to reach within a short time.
3. A large portion (between 80-100%) of the respiratory energy released by the produce is dissipated as heat.

The respiratory heat (Q_r) of the produce is assumed to be produced in proportion to the amount of O_2 consumed and CO_2 produced following equation (3) (Toledo *et al.*, 1969; Kader, 1989; Song *et al.*, 2002).

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6\,CO_2 + 6H_2O + 2816\,kJ \quad (3)$$

The respiratory quotient (RQ) is defined as the ratios of the volume of O_2 and CO_2 released (Eq. (1) & (2).

Based on the oxidation of glucose, RQ = 1. However, in reality, other substrates such as organic acids are oxidised together with glucose in the respiration process. If that occurs then RQ can range from 0.7 to 1.3 for aerobic respiration (Kader *et al.*, 1989). Thus, the model based on the oxidation of glucose and the variability of RQ was considered by taking an average value of the oxygen consumption and carbon dioxide evolution (see Eq. (3).

Therefore, theQ_r was calculated using equation (4) based on oxidation of glucose.

$$Q_r = \left(\frac{2818}{6}\right) \times \left(\frac{r_{O_2} + r_{CO_2}}{2}\right) \quad (4)$$

Eqn (4) represent a typical respiration heat evolution rate accepted for the respiration of fresh produce Kays (1991) and that respiration heat can be assumed to be proportion to the amount of O_2 consumed and/or CO_2 evolved as in equation (3) (Kader, 1989).

$$Q_r\, W + hA(T_a - T_p) = TR\, \lambda + WC_p \left[\frac{dT_t}{dt}\right] \quad (5)$$

Equation (5) as described by Kang & Lee (1998) refer to the energy transferred through natural convection from the ambient air and that generated from respiration heat within the produce used for latent heat of vaporisation. In a steady state as stated in the assumptions, respiration heat is based on the initial weight of the product at which external and internal temperature becomes equal. Therefore, equation (5) containing $\frac{dT_p}{d_t}$ become equal to zero yielding the rate of moisture evaporation (g/h) as described in Equation (6).

$$TR = \frac{(Q_r . W_i)}{\lambda} \quad (6)$$

For normal storage of fresh fruits and vegetables, the temperature can be assumed to be at wet bulb temperature, calculated from a psychometric chart (ASAE, 1979; Wills *et al.*, 1989). The convective heat transfer coefficient can be calculated using the empirical formulae of the equation for natural convection of a horizontal cylinder and sphere in a laminar region, respectively (Holman, 1976). Using the method described by Mohsenin (1986), three linear dimensions were measured using Vanier calliper with an accuracy of 0.01 mm, length (L), width (W), and diameter. Water

displacement method was used to estimate the volume (Vm) of fruit and the geometric mean diameter (Dg), sphericity (L) and surface areas (S) were calculated accordingly (Mohsen, 1986).

Figure 5.1 shows the set for measurement of the whole fruits kept in 5L containers for 4 months, for the purpose determining respiration rates. The jars were fitted with a rubber septum where the gas measurement was done using the gas analyser (PBI Dansensor, Ringsted, Denmark) to monitor O_2 consumption and the evolution of CO_2 rates. The system was designed to flush excess gas within (± 0.5% margin). Weight losses during storage were measured periodically (monthly basis) and the mean of eight fruits was obtained to minimise data variability. Transpiration rate was calculated using the method adapted from (Leonardi *et al.*, 2000).

Data analysis

The experimental data obtained was analysed by a one-way analysis of variance (ANOVA) at a 95% confidence interval to evaluate the effect of time and temperature on respiration rate and respiratory quotient (RQ) using Statistical software (Statistical 10.0, Statsoft, USA).

RESULTS AND DISCUSSION

Table 5.1 shows the physiological parameters measured during the storage period. They include discrete measurements needed for the calculation of TR Changes in mass of pomegranate over time are presented in (Figure 5.1.2). Values were normalised with respect to the initial mass of pomegranate (Mi, g) stored at 5 °C, 96% RH for 5 months. The typical cumulative water loss of pomegranate obtained experimentally using the discreet measurements is presented in (Figure 5.3), showing a high degree of linearity $R^2 = 0.96 \pm 0.01$. There was no significant difference between the two treatments (CA1 and CA2) in terms of loss of moisture during the storage period, also confirmed by normalised mass showing lower weight loss during the same period. The changes in mass of pomegranate decreased linearly in both cases with correlation factor (r = 0.94) for the two treatments shown in (Table 5.1.3).

The respiration rate of pomegranate stored under CA1 was $RCO_2 = 6.04$ mmol/kg.h while under CA2, it was $RCO_2 = 15$ mmol/kg.h). The respiratory heat (Qr) in respective atmospheres were 2647.04 J/kg and 3545.81 J/kg, for CA1 and CA2, respectively. The higher Qr generated by CA2 was possibly due to higher respiration associated with stress caused to the fruit on exposure to the CA2 with relatively high amount of CO_2 than CA1 atmosphere. Li & Kader (1989) reported a similar behavioural pattern with exhibiting high respiration rate for strawberries stored under low O_2 and high CO_2 conditions. The authors concluded that the increase and/or decrease in respiration rates (RR) were due to the recovery process. However, in this study, the variance in the RR was not significant

($p<0.05$) to cause a major variation in TR, which lay within 0.37 and 0.51 g/kg.h for fruits stored under CA1 and CA2, respectively. Table 5.1.2 shows the prediction model based on respiratory energy equation as described by Song *et al.* 1995 method using ($TR = \frac{Q_r Wi}{\lambda}$) described in equation (6). Based on the calculation the model gave TR = 0.39 g/kg.h constant throughout the storage period. The graphical representation of TR is presented on (Figure 5.1.3). By way of comparison between the two TRs (model and experimental), the model accuracy was R^2 = 0.97 correctness.

CONCLUSIONS

The TR model based on respiration energy was validated using discrete weight loss methods of determining transpiration rate. The two methods were consistent and accurate with a negligible difference in their R^2 equal to 0.97. Although the RR was 1.3 fold lower in terms of the evolution of CO_2, the ultimate impact on TR was insignificant compared to CA2. The benefit of both CA1 and CA2 is based on achievement of less than 10% weight loss for pomegranate fruit during the entire storage period. Consequently, the kinetic model appeared simpler to calculate TR under non-invasive techniques and less costly to perform than those reported in the literature involving invasive techniques by damaging the fruit.

TABLES AND FIGURES

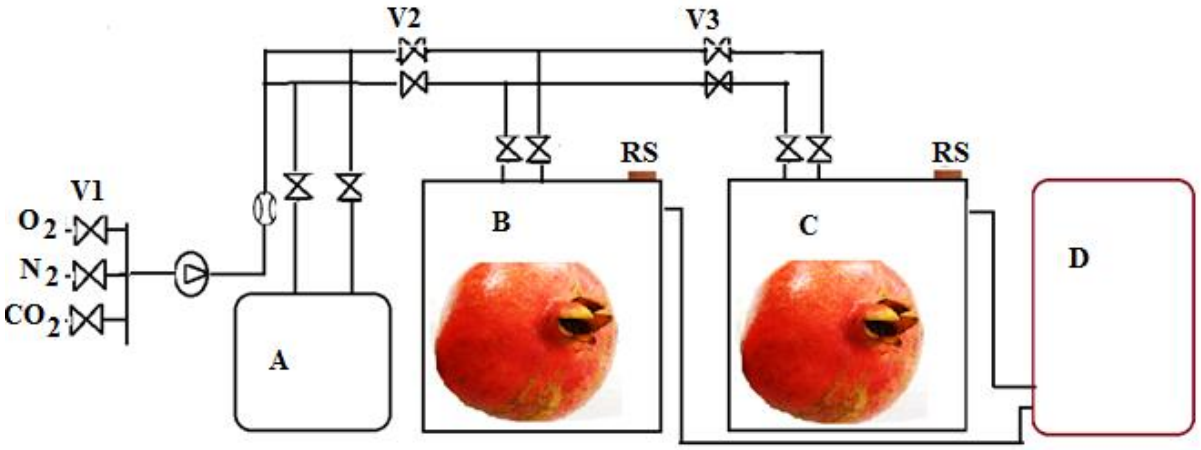

Figure 5.1.1. Schematic diagram of an automated 'CA' system' (Agriculture Research Council-Stellenbosch)

Key

A=Computerised gas analyser;

B=CA1 = (3% O_2 + 6% CO_2)

C=CA2 = (5% O_2 + 14% CO_2)

D= Data acquisition computer (O_2, CO_2, ^oC, % RH monitor)

V_1, V_2, V_3, Open/close valves

RS= Rubber septum, for gas measurement.

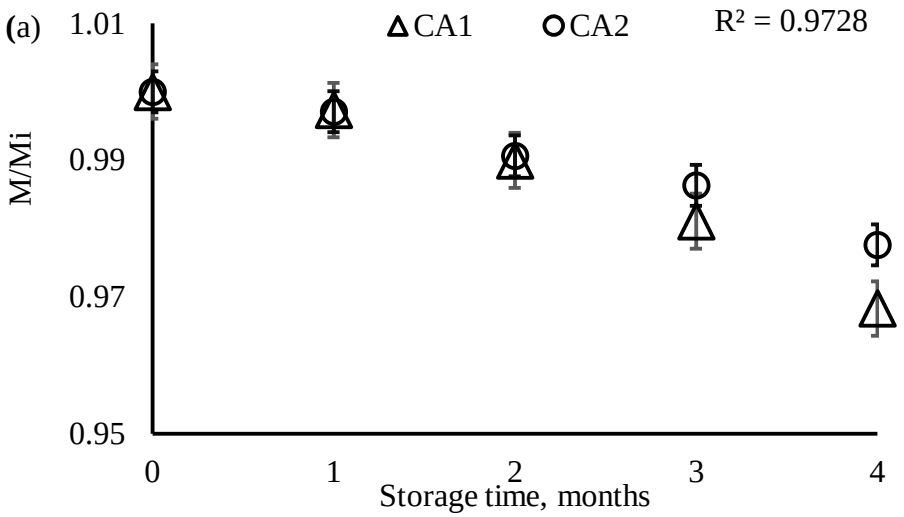

Figure 5. 1.2. Typical changes in mass loss (a) of pomegranate (M, g) over time. Values were normalised with respect to initial mass of pomegranate (Mi, g) stored at 5°C °C, 96% RH for 5 months in 3% O_2 + 6% CO_2. Error bars signify variation at $p<0.05$).

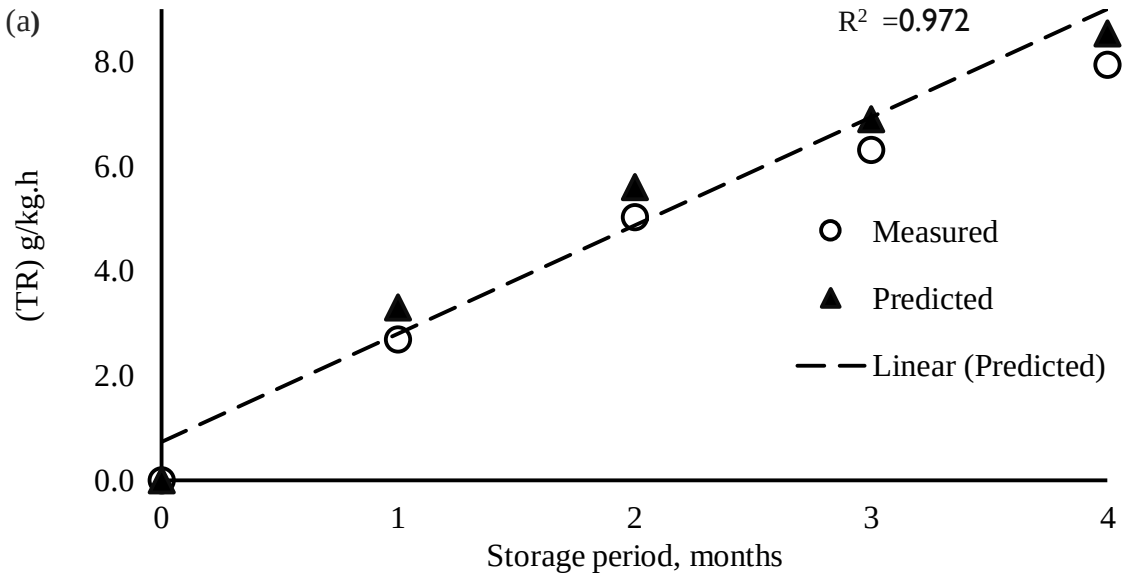

Figure 5.1.3. The cumulative water loss (predicted and measured). Conditions were (3% O_2 + 6% CO_2) at 5 °C at 96% RH.

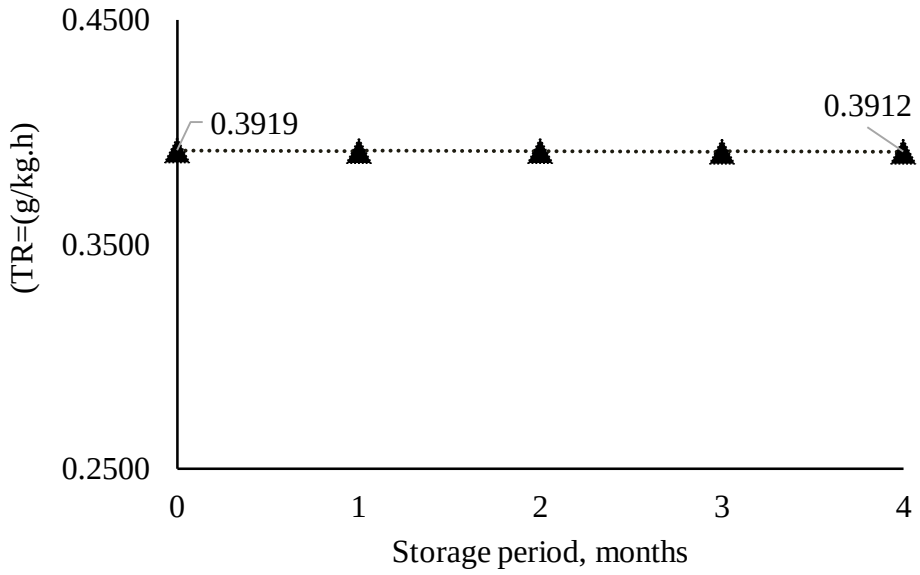

Figure 5.1.4. Graphical representation of predicted transpiration rate based on discrete measurement of physiological loss of weight during storage period calculated based methods available in literature (Caleb *et al.*, 2013).

Table 5.1.1. Physical and physiological characteristics of cv. 'Wonderful' pomegranate at 5 °C

Storage temperature (°c)	3% O_2 + 6% CO_2	5% O_2 + 14% CO_2
Weight (kg)	0.315 ± 5.11	0.327 ± 4.35
Volume (m^3)	0.00032 ±5.2	0.00032 ± 4.8
Transpiration rate (TR=(g/kg.h)	0.37 ± 0.03	0.51 ± 0.06
Rco_2 (mmol/kg. h)	6.04 ± 0.10	15.51 ± 0.12
Ro_2 (mmol/kg. h)	6.67 ± 0.11	14.68 ± 0.12
Latent heat of moisture evaporation (ʎ), (j/kg)	2258	2258
Respiration heat ($\boldsymbol{Q_r}$), (J/kg)	2647.04	3545.81

Table 5.1.2. Predicting transpiration rate using respiratory energy equation as described by Song *et al.* 1995 method using $(TR = \frac{Q_r Wi}{\lambda})$

Month	WI (kg)	Q_r (J/kg)	(ʎ) (J/kg)	Predicted (TR) (g/kg.h)
0	0.334	2647.04	2258	0.391893
1	0.3337	2647.04	2258	0.391635
2	0.3336	2647.04	2258	0.391494
3	0.3335	2647.04	2258	0.391342
4	0.3334	2647.04	2258	0.391213
Mean	0.3334	2647.04	2258	0.391019

Table 5.1.3. Summary staitistics for pearson correlation for 3% O_2 + 6% CO_2 and 5%O_2 + 14% CO_2 storage conditions

	3% O_2 + 6% CO_2	5% O_2 + 14% CO_2
Pearson r	0.9732	0.9704
P value (two-tailed)	0.0011	0.0013
P value summary	**	**
Is the correlation significant? (α=0.05)	Yes	Yes
R square	0.9471	0.9416

Notation

A	Surface area of produce (m^2)
C_p	Specific heat of produce (J/kg "C)
d	Characteristic dimension of fresh produce (m)
h	Convective heat transfer coefficient (J/m' "C h)
Gr	Grashof number
k	Thermal conductivity of air (J/m℃C h)
TR	Rate of water loss (kg/h)
Q_r	Respiration heat (J/kg h)
R_{O_2}	Respiration rate of oxygen consumption (mmol/kg h)
R_{CO_2}	Respiration rate of carbon dioxide evolution (mmol/kg h)
T_a	Ambient temperature (℃)
T_p	Produce temperature (℃)
t	Time (h)
W	Produce weight (kg)
λ	Latent heat of moisture evaporation (J/kg) 2258

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CHAPTER 5.2

5.2 APPLICATION OF THE GENERAL LINEAR MODEL (GML) AND PARETO CHART TO DETERMINE THE BEST STORAGE CONDITION FOR 'WONDERFUL' AND 'BHAGWA' POMEGRANATE CULTIVARS

Abstract

Changes in quality of pomegranate stored in two different 'CA' (3% O_2 + 6% CO_2 and 5% O_2 + 14% CO_2) conditions at 5 and 7.5 °C and 96% RH are reported. The general linear model (GLM) was used to test the effects of independent variables on the output in terms of either minimising and or/maintaining fruit quality during storage. Results show evidence of strong interaction of variables (O_2, CO_2 and Temperature) that influenced physiological (respiration and transpiration rates), external colour, texture and compositional changes (total soluble solids, titratable acidity, firmness, flavour, volatility of the compounds, antioxidant) quality attributes. Using GLM, it was possible to identify optimum storage combinations of treatment (O_2, CO_2 and Temp) which resulted in maximum levels of individual quality attributes than achieve a holistic/ total quality optimisation. In view of this, 'CA' would make a significant contribution to a goal-oriented optimisation strategy. Under this approach, the output would be optimised under defined 'CA' conditions and optimal temperature and relative humidity. In addition, the GLM model explained more than 99.73% of the variance of independent variables.

Introduction

The 'CA' is considered one the novel method used in extending postharvest life of horticultural products. Owing to the ongoing process of imposing restrictions on the use of chemical and fumigant to preserve agricultural produce (Mari *et al.*, 2014) it became imperative to investigate the application of 'CA' technology for storage fresh pomegranate fruit. Elyatem & Kader (1984) reported that pomegranate is highly perishable with a limited shelf life of two months under room air storage at 5 ° For example, the current postharvest and storage method of using MAP (Xtend® film bags bags, StePac, Tefen, Israel) are only able to extend shelf life up to 3 months under cold storage (Artes *et al.*, 1996). However, empirical studies involving 'CA' storage have shown indication of extending shelf life for more 5 months after harvest depending on cultivars and growing conditions (Kupper *et al.* 1995; 1996; Hess-pierce & Kader, 2003; Defilippi *et al.* 2006). The technology relies on lowering respiration rate, ethylene production, and reduces weight loss in pomegranate (Artes *et al.*, 1996;

Nerya *et al.*, 2006; Defilippi *et al.*, 2006). The storage conditions showed a difference in response of fruit to 'CA' storage and that, no specific 'CA' was can be suitable for all all cultivars.

The main concern during postharvest operations is the storage, modification of storage conditions to maintain the quality of pomegranate. To satisfy the industrial and/or consumer desire for the fruit with a maximum nutritional benefit on a competitive market optimal storage conditions should be used (Kader & Rolle., 2004). Pomegranate producer's concerns include delivery of a good quality fruit in addition to extending shelf life for long distant export markets. On the other hand, wholesale and retail market, appearance, firmness and shelf life are important from the point of consumers (Kader & Rolle, 2004). Consequently, to meet these qualities, Kader (2002) described an integrated model for optimisation of production and quality costs and how they relate to production and storage. Sometimes, storage is needed to provoke desired quality changes or maintain specific quality attributes. This current chapter focuses on the optimising the quality of fresh pomegranate using 'CA' storage. Optimisation offers the trader and the consumer a balance between the price and quality of the produce taking into consideration the desired quality. The objective of this study was to optimise the 'CA' storage conditions in order to simultaneously maximise and/or maintain pomegranate quality attributes during storage.

Methodology to optimise storage conditions

The general linear model (GML) and Pareto chart were used to assess optimal levels

$$Y = \mathrm{XB} + U \qquad (1)$$

Where Y is a matrix of series of multivariate measurements, X is a matrix that might be designed matrix, B is a matrix containing parameters that usually to be the estimated and U is a matrix of errors.

Materials and methods

Pomegranate (*Punica granatum* L.) cv. 'Wonderful', harvested manually during commercial harvest period were obtained from Robertson valley farm, Western Cape (33°480 S, 19°530 E), South Africa. Pomegranate fruits were transported to the postharvest laboratory at Stellenbosch and stored at 5 °C before the commencement of the experiment. The individual fruits were removed from Xtend® film bags, placed inside glass jars of about 5L, and equilibrated at 5 °C for 24 hours prior to the experiment.

Experimental 'CA' conditions

Controlled atmosphere conditions were created by flushing out excess O_2 using nitrogen to achieve a desired level of CA1; 3% O_2 + 6% CO_2 and CA2; 5% O_2 + 14% CO_2 at two different temperatures 5 and 7.5 °C in a room conditioned at 95% RH. The 'CA' conditions were held constant throughout the whole time span of the experiment within ± 0.5 % pre-set level. The gases were measured periodically using a gas analyser (PBI Dansensor, Ringsted, Denmark). The gas measurement process involved inserting the syringe through a septum into the 'CA' container /package. Then at touch of a start button, a small amount of gas sample is drawn into the PBI gas analyser. The result appear immediately on the display, giving the instant indication of the gas composition, which passed through the instrument. Test data was used to make a decision to adjust to the respective gases expected 'CA' requirement. Fruits were evaluated for physicochemical properties periodically and lastly at the end of the five months' storage period.

Quality measurements

Physico-Chemical analysis

Measurement of weight loss, respiration, transpiration, colour, and total soluble solids, TA, pH and others compositional changes were performed according to standard methods as described below:

Weight loss (%)

Three pomegranate fruits from each of the five boxes per treatment (total 15) were labelled and their weights monitored throughout the storage period. Weight loss was expressed as a percentage of initial weight.

Physiological disorder

Whole pomegranate fruits were visually inspected for external physiological disorders and internal by cutting to examine for any form of decay as described by Palou *et al.* (2007). The physiological disorders (skin pitching, Mould, scalds and decay) were quantified using a 3-point scale, 0 = none visible; 1 = slight (≤ 25% of the skin); 2 = moderate (26-50% of the skin); and 3 = severe (> 50% of the skin) while fruits with incidence of mould were automatically regarded as spoiled. Treatments in which more than 25% of the stored fruit displayed decay were terminated.

Total soluble solids (TSS), titratable (TA) and pH

The (TSS, TA and pH) were analysed using the methods described by Fawole *et al* (2012; 2013). Arils from six pomegranates per treatment were separately blended and seeds separated from juice from which the TSS, TA, and pH were measured using standard methods described by Fawole & Opara (2013). Total soluble solids were measured at 20ºC using a digital refractometer (Abbe refractometer model 10450, American Optical, Buffalo, NY), and TA was determined potentiometrically using four grams of juice diluted with 20 mL of distilled water and then titrated to an end point of 8.1 - 8.2 using 0.1N NaOH and expressed as percentage of citric acid. The pH was measured at room air by a pH meter (Model 507 Crimson, Barcelona, Spain). All samples were done in duplicate and results expressed as mean standard error (± SE).

Antioxidant properties of pomegranate

The methods for analysis of total phenolic compounds (TP), radical scavenging activity (RSA), Ascorbic acids (AA), Ferric ion reducing antioxidant power (FRAP) will be analysed as described in by Fawole & Opara (2013).

Data analysis

A two-way ANOVA using STATISTICA software (Statistica 13.1, Statsoft, USA, 2016), for the data analysis, the general linear model (GLM) analysis was performed and means were separated using the LSD test at p=0.05. Posthoc test of homogeneity was performed using Levene's test of homogeneity of variance

RESULTS AND DISCUSSIONS

Results show that there is evidence of an interaction between factors (treatments (CA1 x Temperature) and (CA2 x Temperature) (Table 5.2.1). By cross checking with results on Table 2, CA2 –stored fruit had a higher TSS value at 7.5 ^{o}C than 5 ^{o}C, while those packed in CA1, there was no significant difference at the two temperatures (5 and 7.5 ^{o}C). In addition, the Pareto chart (Fig. 5.2.1) shows shown interactions between variables influencing the compositional changes (O_2 or CO_2 (in CAs). This specific observation was confirmed by the GLM results on Fig. 5.2.1 (b) showing the interactive effect of treatments (CA1 and/or CA2) with temperature 5 and 7.5 °C, respectively. In this figure, CA2 had a 3.3% a higher at 7. 5 °C compared to CA1 at 5 °C storage conditions. It can be

confirmed that biological-based systems have a complex time-varying behaviour due to the dynamics systems, which are often uncertain (Straten & Boxtel, 1995). Furthermore, Hess-Pierce & Kader (2003) observed that a 'CA' with 5% O_2 +15% CO_2 (balance N2) was considered as optimal for pomegranates cv. 'Wonderful' stored at 7 °C at 90–95% RH. However, other cultivars such as cv. 'Hicaz' and 'Mollar' had a separate range of gas compositions (Kupper *et al.*, 1995; Artes *et.al.* 1996).

With regard to weight loss, the Pareto chart (Figure 5.2.2 (a) and (b)) all showed lower weight loss under CA2 than CA1 at the same temperature 5 °C, 96% RH. The response could be attributed to higher CO_2 under CA2 with the potential to lower the biochemical activities (respiration and/or transpiration rates) which have a direct impact on the reduction of moisture loss and weight loss. In other words, the respiratory heat (QR) responsible for transpiration of the produce is proportion to the amount of O_2 consumed and CO_2 produced following equation reported by (Sastry *et al.* 1982; Kader, 1989; Song *et al.*, 2002).

$$C_6\ H_{12}O_6\ + 6O_2 \rightarrow 6CO_2 + 6H_2O\ + 2816\ \text{KJ} \qquad (2)$$

The Pareto chart (Figure 5.2.3) showed the effect of temperature, O_2 and CO_2 in influencing weight loss. It was evident that O_2 played a significant role than CO_2 or a combination of (Temperature + O_2) influencing weight loss. The GLM established that the combination of (I) (temp 5 °C + CA1) was less effective in preventing moisture loss compared to CA2 at 7.5 °C possibly for reasons described earlier references (Sastry *et al.*, 1982; Kader, 1989; Song *et al.*, 2002). Moisture losses through transpiration results into fruit shrivel loss of fruit quality and overall nutritional quality (Kader, 2006).

With regard to physiological disorders, pH and antioxidant activity, a similar pattern of interactive effects of temperature and 'CA' variables during storage was observed. These results confirm reports that support the theory of interactive effects of variables and factors to produce an output (compositional changes (Kader, 2006). Many of the biochemical processes within the fruit happen simultaneously and are interrelated in nature (Kader, 1980). The atmosphere with elevated CO_2 potentially inhibits, stimulate, or have no effect on ethylene (C_2H_4) production in fruit that is responsible for senescence in fruits (Kader, 1980). In pomegranate, only trace amounts (very low) nearly 0.01 - 0.1 are produced (Elyatem & Kader, 1984). Similarly, low oxygen (O_2) during storage under 'CA' retards fruit ripening by inhibiting both productions of /and action of C_2H_4.

On the other hand, the O_2 was more effective than temperature alone or in combination Pareto chart (Fig 5.2.2). Saltveit (2001) highlighted natural variability of the fruit, its dynamic response to specific storage conditions that truly render holistic optimisation of 'CA' storage conditions problematic. This study has shown that the goal-oriented approach could appropriately identify optimal storage conditions for specific quality attribute. This method has proved to be efficient in the

identifying optimal storage conditions suitable to achieve the maximum desirable market driven quality attributes characterised by individualised customer, as reported by Schlick *et al.* (2014). For example, total soluble solids were higher under CA2 (5% O_2 + 14% CO_2) at 7.5 °C storage temperatures, yet such a combination may promote the development of undesirable volatile compounds after long storage duration. Furthermore, on Fig. 5.2.3, under CA2, the RSA was significantly higher than under CA1 at 5 °C. These results are in agreement with Willcox *et al.* (2006) who studied the goal-oriented model-constrained optimisation for reduction of large-scale systems. They demonstrated the ability to target a particular output of interest.

With regard to Vitamin C, the results of this study are not in agreement with those reported by Kupper *et al.* (1995) an insignificant change occurred in 'CA' with a low concentration of CO_2. The authors investigated the effect of two 'CA' combinations on pomegranate 'Hicaz' under (1.5% O_2 + 3% CO_2 and 3% O_2 + 6% CO_2, respectively. They observed a minimal loss of vitamin C in 'CA' with lower CO_2 concentration. Considering that, the market is characterised by individualised customer wishes whose expectations are differently, a goal-oriented approach could be the desired optimisation strategy.

CONCLUSIONS

Considering the biological response of the pomegranate fruit as a function of operational variables, GLM and Pareto analysis revealed a significant interaction of variables (CA1 or CA2) and temperature (5 or 7.5 °C). Consequently, the impact resulted in several compositional changes in pomegranate juice. In addition, the dynamic output response in terms of the quality attribute was typically non-linear. In view of this, the most reliable and economic optimal model can be obtained using a goal-oriented output (quality) than holistic approach with negligible compromise in some quality attributes. However, the selection of the appropriate snapshot set of quality attribute remains demand-driven and an open issue. Based on the goal-oriented approach the optimal conditions that have the potential to address the critical aspects of the physiological quality and weight loss is CA2 at 7.5 °C. An improved knowledge of the effects of different 'CA' combinations, temperatures, and the physiological responses in terms of respirations, and the respective interactions would lead to designing appropriate postharvest storage method for fresh pomegranate.

TABLES AND FIGURES

Table 5.2.1. Univariate tests of significance for percentage TSS sigma-restricted parameterisation

Effect	MS	SS	DF	F	P
Intercept	2740.48	2740.48	1	75035.22	0.000
Temp	9.34	9.34	1	255.83	0.000
Treatment	6.24	6.24	1	170.94	0.000
Temp x Treatment	10.81	10.819	1	295.95	0.000
Err	0.04	0.29	8		

Where; Treatment=CA1; (3% O_2 + 6% CO_2), CA2; (5% O_2 + 14% CO_2, Temperature=5 and 7.5 °C

Table 5.2.2. Descriptive statistic for TSS, LSD test; variable homogenous groups, alpha p= .05.

Temperature (°C)	Treatment 'CA'	Mean (TSS) °Brix	b	a	c
5	CA1	15.90	****	****	
	CA2	12.56			****
7.5	CA1	15.70	****		
	CA2	16.22		****	

Where; Treatment=CA1; (3% O_2 + 6% CO_2), CA2; (5% O_2 + 14% CO_2, Temperature=5 and 7.5 °C

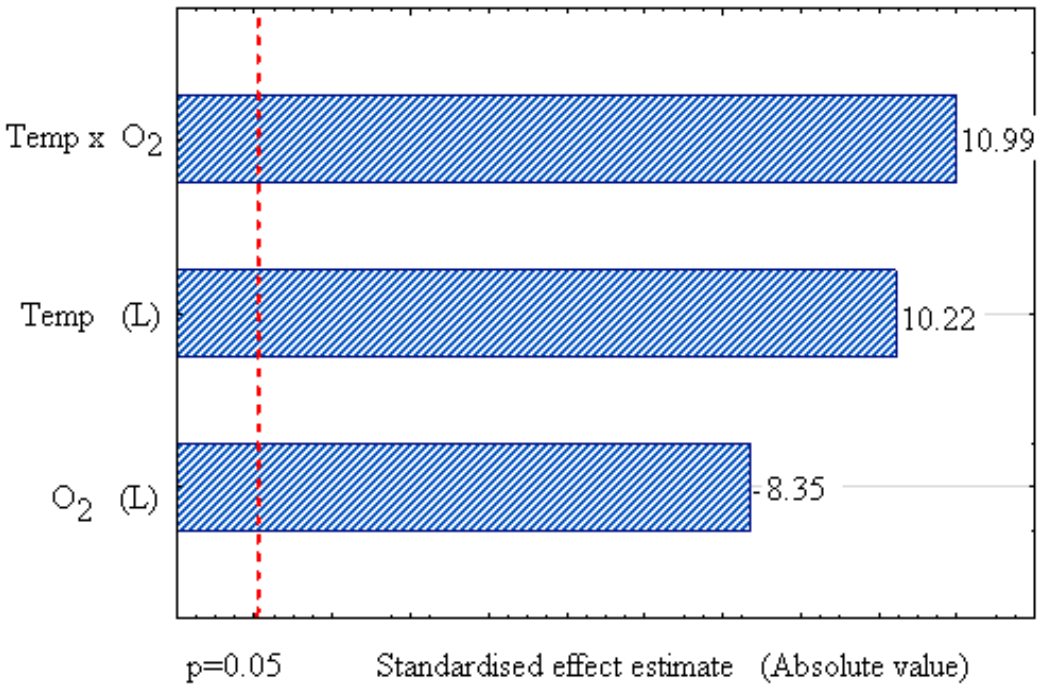
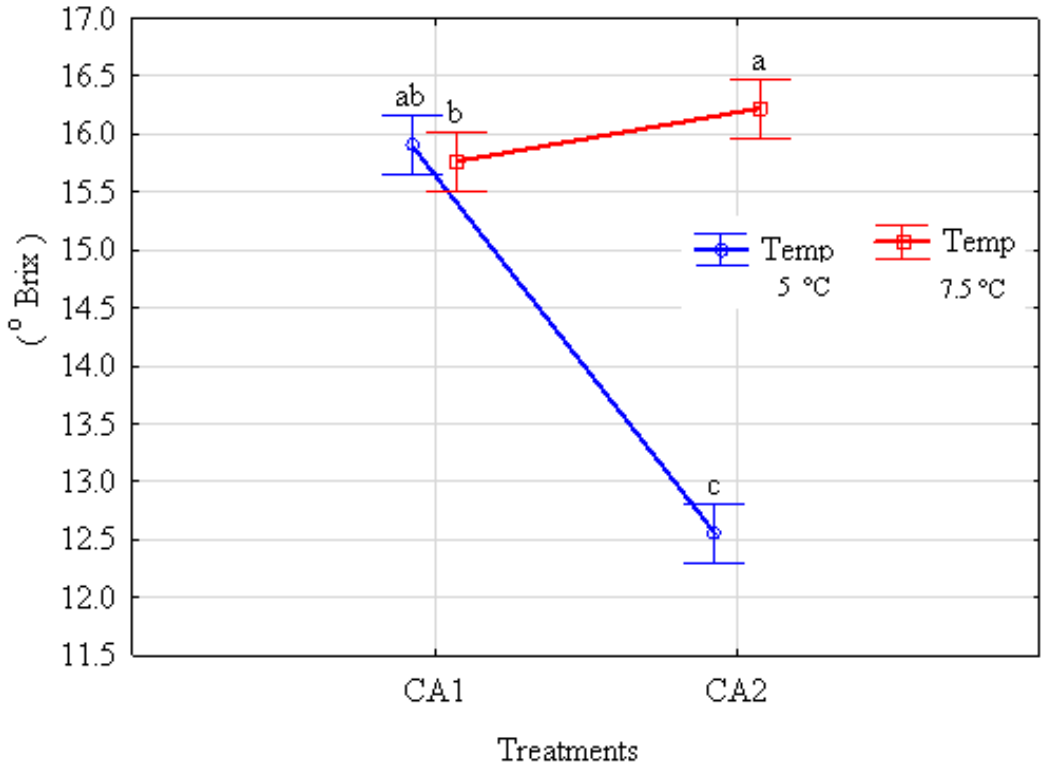


Figure 5.2.1 (a). Pareto chart showing the effect of temperature and O_2/CO_2 on TSS of 'CA' stored 'Wonderful' pomegranate, at 7.5 ºC.



5.2.2 (b). Interaction between treatments (CA1 or CA2) at (5 or 7.5 ºC). Different letters indicate significance at p=0.05. Where; Treatment=CA1; (3% O_2 + 6% CO_2), CA2; (5% O_2 + 14% CO_2

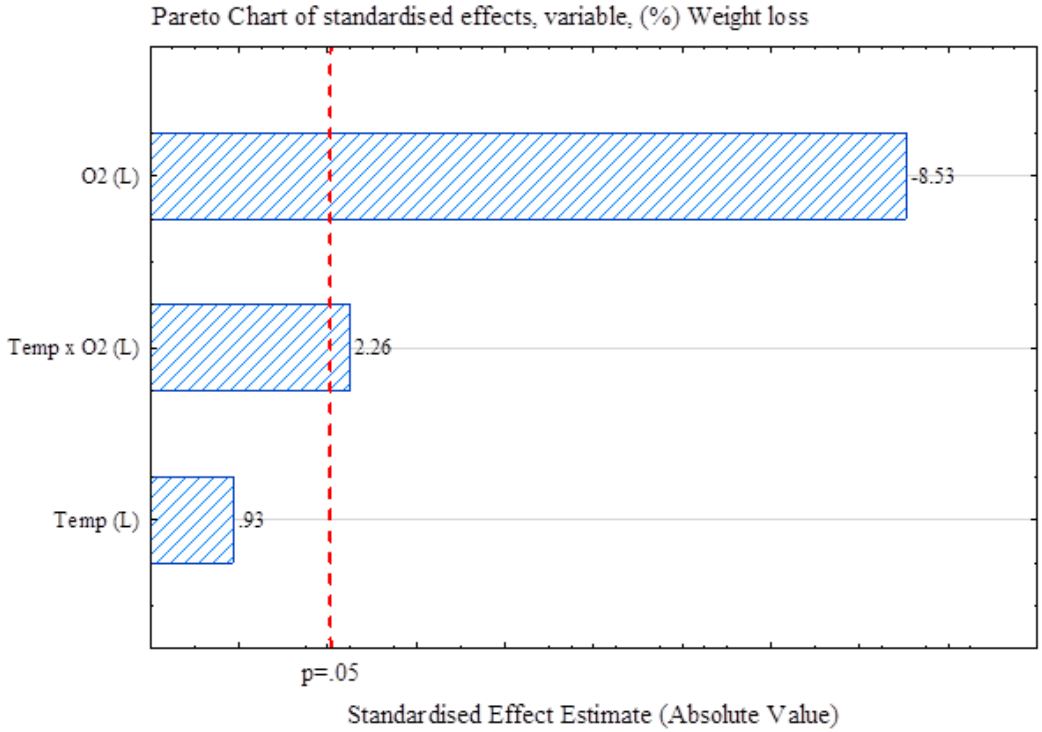


Figure 5.2.3 (a). Pareto chart showing the effect of temperature and O_2/CO_2 on weight loss (percentage) of 'CA' stored pomegranate 'Wonderful'. Different letters indicate significance at p=0.05.

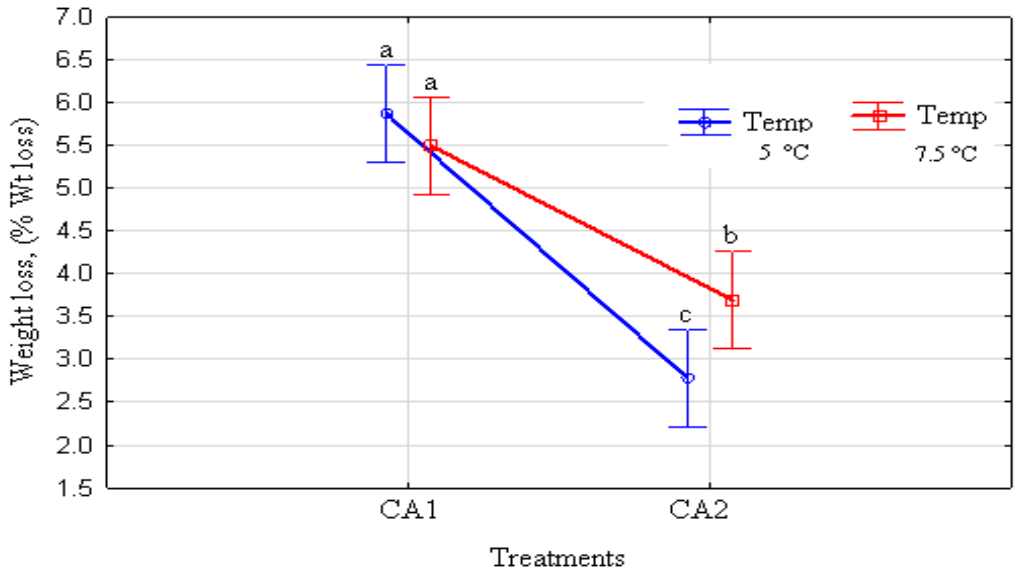


Figure 5.2.4 (b). Interaction effect of (Treatment x Temperature) on weight loss (percentage) of 'CA' stored pomegranate cv. 'Wonderful'. Vertical bars and different letter denote significance difference at p<0.05. Where; Treatment=CA1; (3% O_2 + 6% CO_2), CA2; (5% O_2 + 14% CO_2

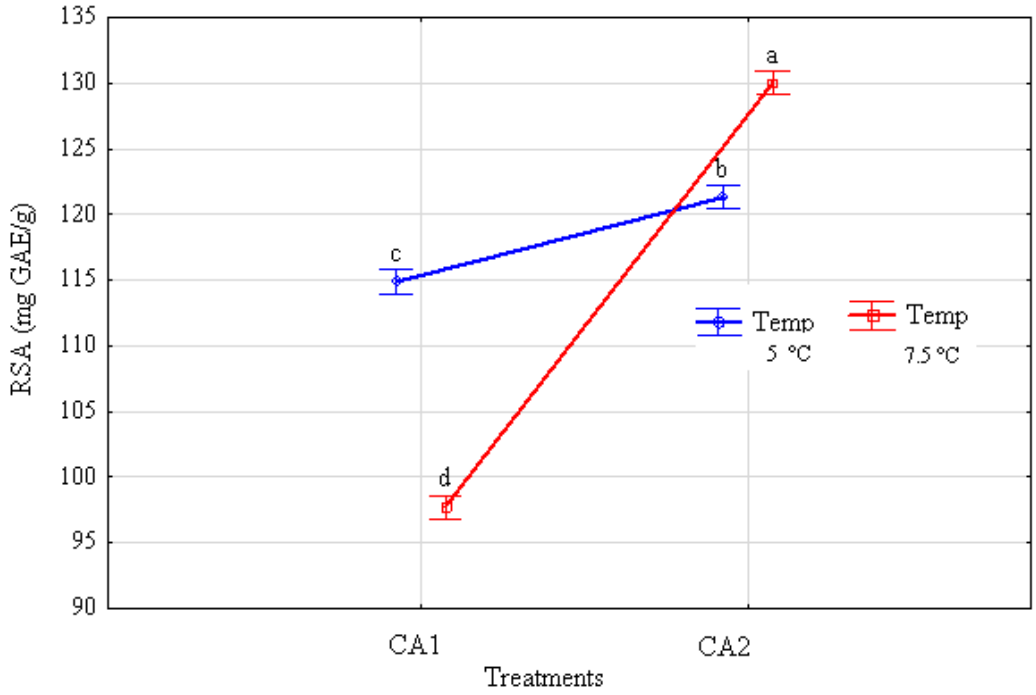


Figure 5. 2.5. Interaction effect of (Treatment x Temperature) on radical scavenging activity (RSA) (mg GAE/g) for 'CA' stored cv. 'Wonderful' pomegranate. Vertical bars and different letter denote significance difference at $p<0.05$.

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CHAPTER 6

CHANGES IN VOLATILE COMPOSITION AND SENSORY QUALITY OF SOUTH AFRICAN POMEGRANATE 'WONDERFUL' STORED UNDER CONTROLLED ATMOSPHERE

Abstract

A study was conducted to evaluate the influence of 'CA' and temperatures on the volatile composition and sensory quality of South African grown cv. 'Wonderful' pomegranate. The 'CA' conditions (CA1; 3% O_2 + 6% CO_2 and CA2; 5% O_2 + 14% CO_2) and temperatures (5, 7.5 and 10 °C) were tested. Sampling was done on a monthly basis, followed by three-day storage in the room air before further analysis. Evaluation of volatiles compounds was done using a gas chromatography–mass spectrometry (GC-MS), while the descriptive sensory analysis was conducted using 5-point hedonic scale by 10 semi trained panellist from Sarchipostharvest group, who are regular consumers of pomegranate fruit. The results showed significant changes in the volatile composition of 'CA' stored pomegranate compared to fruits at baseline in room air. The monoterpenes (alpha- terpinene, gamma-Terpinene), the alcohol group (ethyl alcohol, 1-butanol, and 1-hexanol), aldehyde group (hexanal, acetyl aldehyde); acids and Esters were predominant in all treatments as storage period progressed. These volatile compounds had a significant influence on sensory attributes contributing to an approval score rating of slightly above 50% for pomegranate floral tastes for fruit stored fruits. The decrease in preference was attributed to increased development of ethyl alcohol particularly under CA2 at all temperatures. The Pearson correlation of ($r > 0.7$) showing the association of the build-up of the alcohol /aldehyde aroma compounds and declining flavour liking demonstrated by the descriptive sensory panellists.

Keywords: Aroma, flavour, fruit, postharvest.

Introduction

In the recent decade, pomegranate has gained interest among consumers due to its valuable high nutritional and health benefits. Various studies have shown evidence of these bioactive compounds in improving health and disease prevention (Stover & Mercure, 2007; Viuda-Martos *et al.*, 2010). In addition, numerous research on physiological and quality response of various storage methods such as modified atmosphere (MA) and 'CA' have been reported on pomegranate (Artes *et al.*, 1996; Nanda *et al.*, 2001, Hess-Pierce and Kader, 2003; Defillipi *et al.*, 2006). To this effect, selection of pomegranate cultivars and the global production has increased (Viuda-Martos *et al.*, 2010; Raymond, 2011). However, no literature is available on the effect of 'CA' on aroma and volatile compounds for pomegranate (Caleb *et al.*, 2015); even though empirical studies have shown, that 'CA' can extend shelf life for 6 months after harvest (Kupper *et al.*, 1996; Defilipi *et al.*, 2006). Recently, Mayuoni-Kirshibaum *et al.* (2013) evaluated the impact of MAP on the volatile composition of pomegranate in which the internal MAP atmosphere was (15% O_2 + 5% CO_2) gas composition. They observed numerous changes in volatile composition during storage. Given that 'CA' can extend shelf life, it remains deficient in the area of aroma and health-related attributes, which consumers perceive as a reason to buy the product (Kader, 1989; Moser *et al.*, 2011). With the increasing evidence that consumer choices are based on aroma and volatile compounds (Thilmany *et al.* 2008). Therefore, the objective of this study was to evaluate the changes in volatile composition and sensory quality of 'CA' stored pomegranate grown in South African.

Material and methods

Materials

Pomegranate (*Punica granatum* L.) fruits were purchased from a commercial packhouse in Wellington, Western Cape, South Africa. The fruits were packed into Xtend® film bags (StePac Ltd., Tefen, Israel) immediately after sorting and transported to the University of Stellenbosch. The fruits were stored in three different conditions, CA1; 3% O_2 + 6% CO_2 and CA2; 5% O_2 + 14% CO_2 and in room air (RT) at 20, 5, 7.5, 10 °C, conditions. Fruits were then stored for three months during which sampling was done on monthly basis followed by holding fruit for three more days under RT at 21 °C before analyses of volatile composition.

Methods

Chemical analysis

Three fruits were cut using a knife to open and arils were separated manually, placed in a blender to extract pomegranate juice (PJ). Total soluble solids (TSS) content in the juice was determined using a digital refractometer (Atago, Tokyo, Japan), and acidity percentages were measured by titration to pH 8.3 with 0.1 M NaOH using an automatic titrators (Metrohm, Herisau, Switzerland). Each measurement included three replications, each using juice collected from three fruit.

Extraction and analysis of aroma volatiles

Approximately 10 mL of juice was added to an equal volume of 30% (w/v) NaCl to inhibit enzymatic degradation. Internal standard 50 µL of 3-Octanol (Sigma-Aldrich, St. Louis, MO, USA) was added to the vials and stored at 4 °C pending analysis. The aroma volatiles were trapped and extracted from the vial headspaces an SPME method (Melgarejo *et al.* 2011; Mayuoni-Kirshinbanum *et al.* 2012). The vials equilibrated for 10 min at 50 ºC in the CTC auto sampler incubator. After equilibration time, a 50/30 µm three phase fibre coated with divinylbenzene/-carboxen/-polydimethylsiloxane (needle size 23 ga, Stable Flex, 57298-U Supelco, Sigma-Aldrich) was exposed to the headspace for 20 min at 50 ºC. The samples were equilibrated for 5 min in a 40 °C water bath, followed by an additional 25 min stirring. Desorption of the volatile compounds from the fibre coating was carried out in the injection port of the gas chromatography-mass spectrometry (GC). After extraction, desorption of the volatile compounds from the fibre coating was carried out in the injection port of the gas chromatography-mass spectrometry (GC-MS) during 2 min in split less mode and then 8 min in split mode to clean fibre.

The volatile compounds were separated in the gas chromatograph using Agilent 6890 N (Agilent, Palo Alto, CA), coupled with an Agilent mass spectrometer detector (5975 MS, Agilent, Palo Alto, CA). The GC-MS system was equipped with a Rtx®-5Sil MS, with a 95% polydimethyl siloxane/ 5% polydiphenyl siloxane stationary phase and the dimensions were 30 m length; 0.25 mm inner diameter; and 0.5 µm film thickness. Analyses were carried out using helium as a carrier gas with a flow of 1.2 mL min^{-1}. The oven temperature was as follows: 40 ºC for 2 min; and then ramped up to 250 ºC min^{-1} and held for 5 min. The MSD was operated in full scan mode and the ion source and quadruple were maintained at 240 ºC and 150 ºC, respectively. The transfer line temperature was maintained at 280 ºC. Using the GC- MS analyses, chromatographic peaks for different volatiles were

identified by comparing retention times (RTs) of each spectrum with the National Institute of Standards and Technology (NIST) database.

Sensory evaluations

The descriptive sensory quality evaluation of 'CA' -stored pomegranate arils was performed using a recommended method ((Lawless & Heymann, 1999). Arils separated from six different fruits were placed in plastic cups identified by randomly assigned three-digit codes. The descriptive analysis of sensory attributes included (sweetness, juiciness, colour, sour, bitterness, floral taste, alcoholic taste, aril hardness and overall taste). The evaluation was performed based on a five – point hedonic scale ranging from 1 = strongly dislike, 2= dislike moderately, 3= neither like nor dislike, 4= like moderately and 5= strongly like. The experiment was replicated twice.

Data analysis

A one-way analysis of variance (ANOVA) was done using Statistica software (Statistica 13.1, Statsoft, USA, 2016). The means were separated using the LSD test at p=0.05. Posthoc test of homogeneity was performed using Turkey's Post hoc tests.

RESULTS AND DISCUSSION

The compositional changes in pomegranate quality are presented in (Tables 6.1, 6.2, 6.3). Results showed that the chemical qualities of fruit were affected by the 'CA' condition, temperature and duration. There was a slight decrease in TSS was across all treatments during the during the three-month storage period. Likewise, the acidity (citric acid, malic and tartaric), fluctuated during the storage period. Overall, the mean decrease in acids were approximately 0.23 (%) for (citric, malic and tartaric acids) by the end of third months. It is evident that the changes had an effect on sugar to acid ratio and possibly influenced the overall sweetness of the juice and volatile composition. The changes in TSS across all storage conditions are in agreement with literature values (Kader *et al.* 1984; Al-Mughrabi & Bacha, 1995). The authors attributed the compositional changes to biochemical activities occurring in the cell wall structure as reported by Zhang & McCarthy (2013). The author consolidated their argument that 'CA' influenced the migration of water in and out of the vacuole at the early stage and/or back to the vacuole in the later stage of storage thus inducing changes in total soluble solids.

The aroma volatile compositions of 'CA' -stored pomegranate are presented (Tables 6.4 and 6.5). The results show that 'CA' , temperature and duration had a significant ($P<0.05$) influence on the

development of volatiles compounds. Aldehydes characterised with the fruity, green, pungent flavour) were predominant in fruit juice at baseline and during the subsequent storage duration under 'CA' at 5 °C. The alcohols groups characterised with a minty, ethyl alcohol or resin flavour) was present under CA2 at temperatures 7 and 10 °C. Monoterpenes (α- pinene, β.-pinene, β myrcene, limonene) with a characteristic (Berry, lemon, vegetable, woody, pepper, woody, pine, sweet, balsamic, plastic, mild, citrus, sweet, orange, lemon were among the volatiles in 'CA' -stored fruit. These compounds were also reported in studies under MAP storage (Mayuoni-Kirshinbaum et al. 2012; Caleb *et al.*, 2013; Hamouda *et al.*, 2014). The esters and the acids with a combined nutmeg, floral, and fruit aroma were also present in slightly high levels in the first one month under both CA1 and CA2 but decreased in the third months. However, the same compounds were relatively low at 7.5 ºC while the levels of acetyl acetate were significantly higher at 10 ºC at CA2. The group of monoterpines composed of terpinene derivatives (α- pinene, β.-pinene, β -myrcene and limonene) as the main compounds. These volatile compounds are known to contribute to the citrus, fruity, sweet, balsamic, and musty associated with the natural pomegranate flavour. The low levels of alcohols groups combined with monoterpenes (alpha-terpinene, gamma- Terpinene) observed in 'CA' at 5 ºC were associated with the herbaceous, citrus flavour and insignificant levels of alcohol groups compared to CA2 which had a moderately high level of the same compounds. No chilling injuries were observed during the storage period. As the storage duration exceeded beyond 3 months to 5 months under CA2, over 60% of fruits were characterised by an increase of alcohol group composed of (ethyl alcohol, 1-hexanol and 1- butanol) under CA2. Previous studies under 'CA' with 10-15% CO2 showed an increase in the intensity of off-flavour that were attributed to anaerobic respiration (Hess-Pierce & Kader, 1984; Defillipi et al., 2006; El Hadi *et al.*, 2013). The reduction of the TSS and TA can be associated with a reduction in the sensations one feels on the tongue: sweet, salty, acidic or bitter. The changes in acidity were similar to those reported by Ratore *et al.* (2007) who studied the effect of storage on physiochemical composition and sensory properties of mango. They attributed the decline in acidity to the susceptibility of citric acid or oxidative destruction and activity of citric acid glycosylase or conversion into sugars. Hess-Pierce & Kader (2003) also described similar patterns of TA for a fruit stored in room air. The sensory acceptance test yielded a 60% liking (3 scores out of 5) or flavour preference for CA2 stored pomegranate. The results are in agreement with Kader *et al.* (1977), showing a drop in sugar/acid ratio coupled with the development of flavour and aroma compounds responsible for the decline in preference. Furthermore, an observed development of (alcohol and aldehyde) groups detected by GC-MS, also contributed to some off odour detected by the panellist. The radar plots (Figures 6.1 and 6.2) presented some level of floral tests (2 – 3 scores out of 5 maximum), responsible for the 60% acceptance of (sweet to sour tastes). By comparison, of the radar plots, 'CA' -stored fruit at 7.5 °C had a significantly high level of ethanol

related volatiles depicted on close to the 90°axis for CA2. It is generally known that the 'flavour-life' of fruit is often shorter than their overall 'storage life', as determined by the external visual quality of the produce (Baldwin *et al.*, 2007; Kader, 2008). The radar plot and biplots on (Figure 6.1 and 6.2) show the sensory response of evaluators. Overall flavour profile ranged within the mean 2-3 scores on a five-point hedonic scale signify a 40 - 60% liking of the fruit stored under CA1 and CA2 at 5 °C storage conditions. Fruits stored under CA2 at 7.5 °C were on average rated from one to four, depending on the quality attribute. For example, the floral taste, juiciness and sweetness scale lay between three and four scores. The biplot Figure 6.2 (a) segmented related sensory responses. The repeated sensory tests showed a similar pattern of results as shown on the principal component analysis (PCA) using eigenvalues >one. Based on the eigenvalue greater than one, it is realistic to accept that the sensory scores on the component were reliable to segment according to the factors and quality. This is because the PCA data sets yielded two principal factors (F1 and F2) with eigenvalues >1, explaining more than 73% of the total variance of (F1) factors accounted for over 73% of the total variance between both CA1 and CA2.

CONCLUSIONS

The interaction between 'CA' and storage temperature was highly significant $p<0.05$ in influencing both chemical compositional changes and aroma volatile compounds in pomegranate. Although incidences of cumulative aldehydes and alcohol groups were observed, the fruit retained nearly 60% of the monoterpenes compounds responsible for the natural sweet pomegranate flavour, balsamic and citrus-like fruit flavour. This observation correlated well with the sensory quality perception of with Pearson correlation factor of $r >0.7$. However, the prolonged storage under 'CA' conditions beyond three moth's storage period resulted in an increase of alcohol and aldehyde groups responsible for off-flavour odours. By comparison, CA1 had slightly low levels of alcohol and aldehyde groups. This study has provided a foundation useful for industrial purposes, as well as the development of optimal postharvest handling and processing of pomegranate grown in South African.

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TABLES AND FIGURES

Table 6.1. Characteristic of 'CA' -stored pomegranate fruit at 5 and 21 ºC

Treatment	Storage duration (months)	Temp. °C	TSS °(Brix)	TSS: acid ratio	Citric acid %	Malic acid %	Tartaric acid %
NA	0	21	17.0 a	12.4b	1.37a	1.43b	1.61b
CA1	1	5	15.8b	9.6c	1.64a	1.72a	1.93a
	2		16.7a	13.8a	1.21b	1.26b	1.41b
	3		16.5a	10.7b	1.54a	1.61a	1.80a
CA2	1	5	15.8b	12.3b	1.27b	1.33b	1.49b
	2		15.4b	11.0b	1.40a	1.46b	1.64b
	3		16.0b	11.4b	1.40a	1.47b	1.64b

Where; RT= room air at baseline and TSS = Total soluble solids. Mean data presented in each column followed by different letter (s) are significantly different ($p < 0.05$) according to Turkey's Post hoc tests.

Table 6.2. Characteristic of 'CA' -stored pomegranate fruit juice at 7.5 and 21 ºC

Treatment	Storage duration (months)	Temp. °C	TSS °(Brix)	TSS: acid ratio	Citric acid %	Malic acid %	Tartaric acid %
RT	0	21	17.0 a	12.4b	1.37a	1.43b	1.61b
CA1	1	7.5	16.2b	10.3b	1.57b	1.64b	1.84a
	2		15.0b	13.6a	1.11c	1.16c	1.30b
	3		14.8b	8.1c	1.82a	1.91a	2.14a
CA2	1	7.5	15.9b	9.20c	1.73b	1.81a	2.02a
	2		16.3b	10.9b	1.49b	1.56b	1.75b
	3		15.6b	13.5a	1.16c	1.22c	1.36b

Where; RT= room air at baseline and TSS = Total soluble solids. Mean data presented in each column followed by different letter (s) are significantly different ($p < 0.05$) according to Turkey's Post hoc tests.

Table 6.3. Characteristic of 'CA' -stored pomegranate fruit juice at 10 and 21 °C

Treatment	Storage period (months)	Temp.°C	PH	TSS (°Brix)	TSS/Acid ratio	Citric acid %	Malic acid %	Tartaric acid %
RT	0	21	3.5	17.0a	12.4b	1.37a	1.43a	1.61a
CA2	1	10	3.4	15.6b	14.6b	1.07b	1.12b	1.25b
	2	10	3.5	15.6b	15.3a	1.02b	1.12b	1.25b
	3	10	3.6	15.0b	14.0b	1.07b	1.01b	1.10b

Where; CA2 = 5% O_2 + 14% CO_2, storage temperature at 10 °C, RT= room air at baseline and TSS = Total soluble solids. Mean data presented in each column followed by different letter (s) are significantly different ($p < 0.05$) according to Turkey's Post hoc tests.

Table 6.4. Principal component analysis; Eigenvalue value storage conditions at (a) 5 °C and (b) at 7.5 °C

(a)	*F1*	*F2*	*(b)*	*F1*	*F2*
Eigenvalue	7.8	2.2		7.33	2.23
Variability (%)	78.2	21.8		73.34	22.33
Cumulative %	78.2	100.0		73.34	95.67

Table 6.5. Characteristic of pomegranate juice extracted from fruit stored in CA1 (3% O_2 + 6% CO_2 and CA2 (5% O_2 + 14% CO_2) at 5 and 7.5 °C

Storage (months)	0	1		2		3		1		2		3	
Treatment	RT	3% O_2 + 6% CO_2		3% O_2 + 6% CO_2		3% O_2 + 6% CO_2		5% O_2 + 14% CO_2		5% O_2 + 14% CO_2		5% O_2 + 14% CO_2	
Temp. °C	21	5	7.5	5	7.5	5	7.5	5	7.5	5	7.5	5	7.5
TSS °(Brix)	17.0a	15.8b	16.2b	16.7a	15.0b	16.5a	14.8b	15.8b	15.9b	15.4b	16.3b	16.0b	15.6b
TSS: acid ratio	12.4b	9.6c	10.3b	13.8a	13.6a	10.7b	8.1c	12.3b	9.20c	11.0b	10.9b	11.4b	13.5a
Citric acid %	1.37b	1.64a	1.57a	1.21b	1.11c	1.54b	1.82a	1.27b	1.73a	1.40a	1.49b	1.40b	1.16b
Malic acid %	1.43b	1.72a	1.64b	1.26b	1.16c	1.61a	1.91a	1.33b	1.81a	1.46b	1.56b	1.47b	1.22c
Tartaric acid %	1.61b	1.93a	1.84a	1.41b	1.30b	1.80a	2.14a	1.49b	2.02a	1.64b	1.75b	1.64b	1.36b

Where; CA1 = 3% O_2 + 6% CO_2 and CA2= 5% O_2 + 14% CO_2; and RT = room air at baseline, 5 and 7.5 °C. TSS = Total soluble solids. Mean data presented in each row followed by different letter (s) are significantly different ($p < 0.05$) according to Turkey's Post hoc tests.

Table 6.6. Effect of storage conditions CA1 and CA2 on volatile composition in cv. Pomegranate stored at 5 °C

	Treatment	RT	3% O_2 + 6% CO_2			5% O_2 + 14% CO_2			Descriptor	References
	Storage (Months)	0	1	2	3	1	2	3		
Classes	Volatile comp'd	PA	PA	PA	PA	PA	PA	PA		
Aldehyde	Hexanal	1.5	1.8	0.9	2.7	1.5	2.0	2.9	Grass, tallow, fat	Vázquez-Araujo *et al.* (2011).
	Acetal aldehyde	2.3		7.1	2.8	n. d	9.5	3.7	Pungent	Mayuoni-Kirshinbaum & Porat (2014)
Monoterpenes	Alpha- Pinene	2.1	0.2	0.7	0.3	0.7	0.9	n. d	Pine, citrus	Vázquez-Araujo *et al.* (2011).
	Beta. -Pinene	0.8	0.9	0.3	0.8	0.8	0.9	0.4	Musty, pine	
	Beta - Myrcene	0.1	0.1	0.1	0.1	0.2	0.1	0.1	Sweet, balsamic	Melgarejo *et al.* (2011); Mayuoni-Kirshinbaum & Porat (2012.
	Limonene	0.4	0.8	0.3	0.3	0.3	0.3	0.3	Citrus-like. fruity	
Alcohol	Ethyl alcohol	7.8	8.6	18.6	31.8	17.4	25.3	17.7	Minty ethyl alcohol	Melgarejo *et al.* 2011; Caleb *et al.* (2013).
	1-Hexanol	1.9	1.6	0.2	0.6	0.1	0.6	0.7	Resin, flower, green	
	1 Butanol	n. d	n. d	1.7	0.4	0.1	0.5	0.3		
Acids	Acetic acid	0.2	0.2	4.3	4.5	1.9	4.3	0.8	Nutmeg, floral	Mayuoni-Kirshinbaum & Porat (2014).
Esters	Ethyl acetate	6.0	5.2	8.5	4.4	5.6	0.2	0.2	Fruity, fruity	Mayuoni-Kirshinbaum & Porat (2014).

Where; PA=peak area, mean of two replications, cells without numeric score (n.d) indicate not detected in that specific sample

Table 6.7. Volatile compounds in cv. Pomegranate stored in CA1 (3% O_2 + 6% CO_2 and CA2 (5% O_2 + 14% CO_2) at temperatures 7.5 ℃ and 10 °*C* temperatures

Treatment		RT	3% O_2 + 6% CO_2			5% O_2 + 14% CO_2			5% O_2 + 14% CO_2		
Temperature		20 °C	7.5	7.5	7.5	7.5	7.5	7.5	10	10	10
Storage (months)		0	1	2	3	1	2	3	1	2	3
Classes	Volatile comp'd	PA	PA	PA	PA	PA	PA	PA	PA	PA	PA
Aldehydes	Hexanal	1.5	0.4	1.2	1.5	1.5	n. d	1.4	1.4	0.5	1.7
	Acetal aldehyde	2.3	n. d	n. d	n. d	3.6	0.2	3.8	n. d	7.8	6.0
Monoterpenes	Alpha- Pinene	2.1	0.8	0.9	0.8	0.6	0.8	0.6	2.0	0.4	0.6
	Beta. -Pinene	0.8	0.04	1.2	0.6	0.6	0.7	0.9	0.8	0.3	0.4
	Beta- Myrcene	0.1	0.2	0.4	2.1	n. d	0.2	n. d	0.2	n. d	0.1
	Limonene	0.4	0.3	0.9	0.2	0.3	0.4	0.2	0.9	0.2	0.3
Alcohols	Ethyl alcohol	7.8	7.5	23.1	14.0	17.3	12.9	25.48	7.7	30.8	26.9
	1-Hexanol	n. d	0.6	0.1	0.7	0.4	0.7	0.8	2.3	0.8	0.5
	1 Butanol	1.9	0.8	1.3	1.2	1.1	1.5	0.3	n. d	n. d	n. d
Acids	Acetic acid	0.2	0.7	0.2	1.0	1.0	1.3	4.5	0.2	7.3	4.0
Esters	Ethyl Acetate	0.1	1.4	n. d	3.8	1.4	n. d	n. d	9.3	8.0	2.9

Where; PA=peak area =Experimental mean of area of two replications, cells without numeric score (n.d) indicate not detected in that specific sample

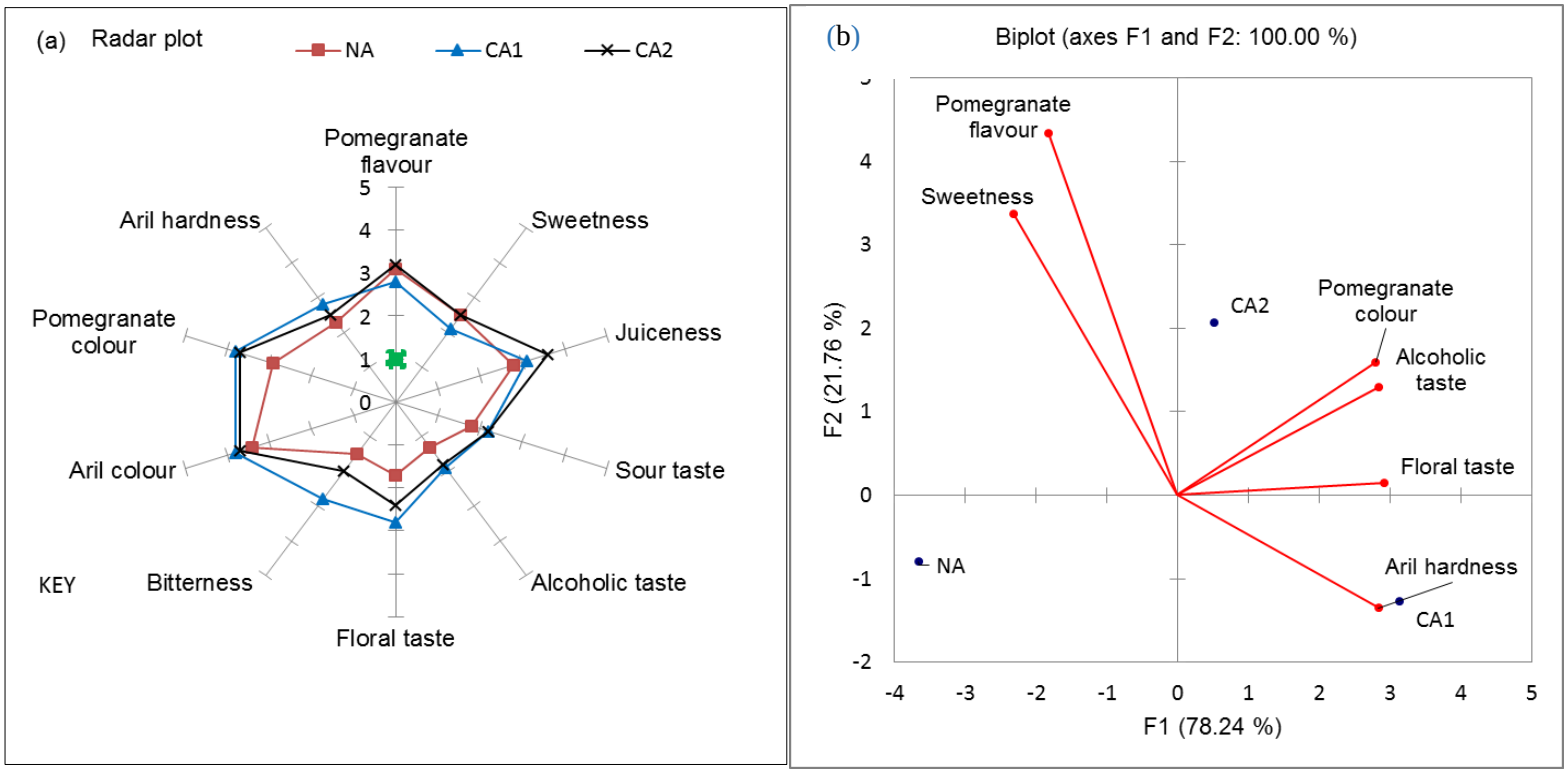

Figure 6.1. Radar plot (a) flavour profiles of cv. 'Wonderful' pomegranate stored at 5 °C under CA1; 3% O_2 + 6% CO_2 and CA2; 5% O_2 + 14% CO_2, respectively. (b) Biplot axes for variable between F1 and F2 for the descriptive analysis of flavour attributes (sweet, sour and bitter), odour (overall pomegranate fruity flavour, off-flavours) and hardness of aril.

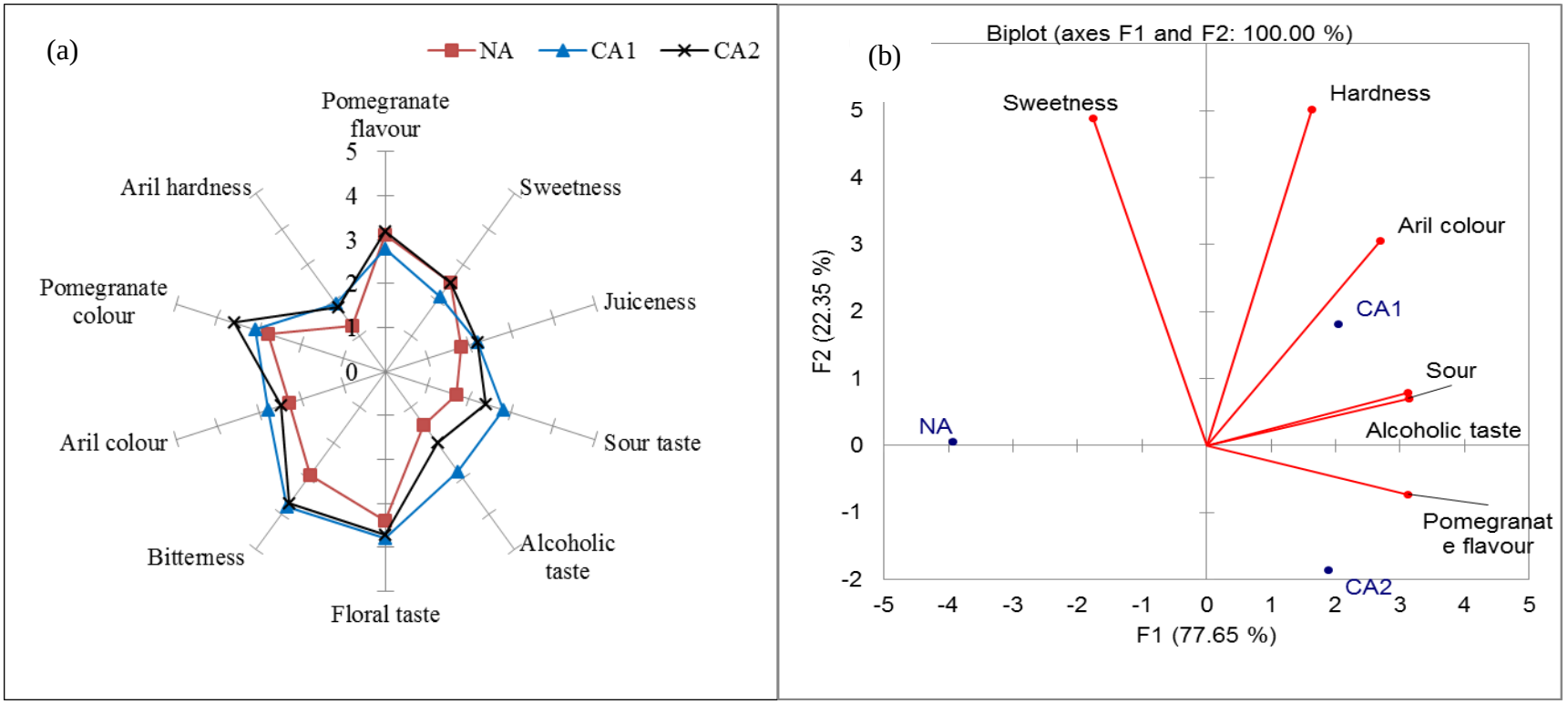

Figure 6.2. Radar plot showing the flavour profiles of cv. 'Wonderful' pomegranate stored at 7.5 °C under controlled atmosphere CA1; 3% O_2 + 6% CO_2 and CA2; 5% O_2 + 14% CO_2, respectively. (b) Biplot axes for variable between F1 and F2 for qualitative descriptive flavour attributes such as taste (sweet, sour and bitter), odour (overall pomegranate fruity flavour, off-flavours) and hardness of aril.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

Pomegranate is a highly perishable fruit with not more than eight weeks' shelf life at minimum safe temperatures 5 °C. The limiting factors to long-term storage include weight loss, shrivelling, husk scald, chilling injuries and loss of colour (Elyatem & Kader, 1984). The physiological deterioration of pomegranate and other perishable fruits increases two to three-fold with every 10 °C increase in storage temperature. The South African pomegranate industry is growing rapidly in response to the aggregate demand for the fresh fruit (local and international) with volumes of over 70% destined for export during the off-season to Europe. The primary aim of this study is to investigate the physiological and quality response of pomegranate fruit under controlled atmosphere storage conditions.

The response of the whole pomegranate cv. ('Wonderful' and 'Bhagwa') to 'CA' condition and storage temperatures (5, 7.5 and 10 °C) at 95% were investigated. The physicochemical properties (respiration and transpiration rates, external colour, texture, total soluble solids, titratable acidity, firmness, flavour, volatility of the compounds, antioxidant properties and modelling the transpiration rates were done. The results showed a strong interaction between the treatments (CA1 and/or CA2) & (temperature). Moreover, it was confirmed that storing fruits in both CA1 (3% O_2 + 6% CO_2) and CA2 (5% O_2 +14% CO_2) at 5°C and /or 7.5°C extended the shelf life of the fruit by two-fold compared to cold storage for both cv. 'Bhagwa' and 'Wonderful', respectively. The variation in response to specific 'CA' influencing compositional changes by either increase and/or decrease or maintain quality attributes merits the need to investigate specific cultivars' response in order to optimise storage conditions for the specific cultivar.

The impact of 'CA' and storage temperatures on antioxidant properties of pomegranate cv. 'Wonderful' were investigated. The interactive effect of temperature and gas composition influenced the measurable changes in bioactive compounds. For example, radical scavenging activity reduced by 1.3 fold lower than the baseline value of (0.8 mg/100 ml A.A, equivalent) at 5 °C storage temperature, whereas the opposite showing a 1.5-fold increase in RSA for pomegranate stored at 7.5 ˚C under the same CA1 (3% O_2 + 6% CO_2) by the end of the third month. The reducing power of pomegranate (FRAP), Diphenylpicrylhydrazyl (DPPH), total phenolic content (TPC) and ascorbic acid were performed by using recommended methods (Makkar *et al*. 2007; Fawole *et al.*, 2012). 'CA' storage conditions (3% O_2 + 6% CO_2) at 5°C had a significant influence on RSA showing a gradual decrease whereas under CA2 (5% O_2 + 15% CO_2 at 7.5°C resulted in a slightly increase RSA during

storage. The phenolic compounds showed a similar response by fluctuating with an increasing and/or decrease depending on the 'CA' storage temperature. There was no significant change in FRAP ($p<0.05$) and the level of ascorbic acid during storage. The results corroborated with those reported for 'CA' stored pomegranate (Kupper *et al*., 1995). In that study, the authors investigated the effect of 'CA' storage of pomegranate 'Hicaz' under' in 1.5% O_2 + 3% CO_2 and 3% O_2 + 6% CO_2 atmosphere, respectively. The study reported that lower CO_2 concentration had little effect on vitamin C levels.

The 'CA' -and storage temperatures had a significant contribution to the development of undesirable volatile compounds. More specifically, CA2 with higher storage temperature (7.5 and 10 °C) resulted in higher level of aldehydes and alcohols products than under CA1 at 5 °C temperature The combined influence on flavour quality and sensory profiles lowered the preference liking to 60% scale. Based on these results, and the information available in the literature (Mayuoni-Kirshinbaum & Porat, 2014), it can be concluded that CA1 (3% O_2 + 6% CO_2) at 5 °C had superior volatile retention capability compared to CA2 which generated higher levels of alcohol and aldehyde over time. Likewise, the reduction in sensory perception of cv. 'Wonderful' pomegranate following prolonged storage under 'CA' conditions is indicative of the need to develop appropriate postharvest 'CA' storage technology suitable to maintain or slow down the severe drop of flavour quality.

Transpiration or loss of moisture from the fruit is one of the major causes of deterioration in fresh horticultural produce after harvest. The water loss results in direct quantitative losses (loss of saleable weight) and biochemical (nutritional) and physiological appearances (wilting and shrivelling). In literature, the use of waxing (surface coating) of through manipulation of storage modified atmosphere (MAP) have been studied (Mirdehghan *et al.*, 2007). The kinetic model developed showed that respiration heat and mass transfer as a function of respiration rate influenced transpiration and weight loss of pomegranate. This was in agreement with the observation made by Li & Kader (1989) who studied the effect of 'CA' storage of strawberries under 'CA' condition. In the particular study fruit held in 15-20% CO_2 significantly lowered respiration rate and reduced weight loss. However, after re removal out of 'CA' and transferred to room air the RR increased with a corresponding Qr (respiratory quotients) by 1.3 fold. This observation highlights the significance of postharvest handling management of fruits after storage

The general linear model and Pareto chart were used to determine the best optimal 'CA' storage conditions. Results showed an interaction of variables (O_2, CO_2 and Temp) that influenced changes in physiological (respiration and transpiration rates, external colour, texture) and nutrient qualities (total soluble solids, titratable acidity, firmness, flavour, volatility of the compounds, antioxidant) quality attributes. Using this method, the p-values was less than any reasonable alpha level, suggesting that evidence exists that the interaction has a significant effect on influencing quality

attributes. In addition, the model explained 99.73% of the variance. The change in respiration quotient for fruits can be attributed to high CO_2 and/low O_2 in the atmosphere, thus triggered changes in the respiration pattern of fruits. No specific atmosphere qualified to maintain all quality attributes singly, due to the interactive nature of variables, and the biochemical and physiological response of pomegranate cultivar. An increased understanding of the effects of different 'CA' combinations, temperatures, and the physiological response in terms of respirations and the respective interactions would lead to improved 'CA' storage of different pomegranate cultivars.

Limitations and future research needs

- The author wishes to acknowledge the limitation of this current study that 'CA' results are only representative of two cultivars 'Bhagwa' and 'Wonderful' procured in one area of (Western Cape) over two seasons. Therefore, results generated at this stage forms a foundation and a basis for future studies.
- The current study endeavoured to optimise storage conditions in the way that focussed attention to the overall quality of pomegranate without critically targeting specific nutrients. However, the findings showed limitation due to the natural variation of biological response of pomegranate to 'CA' storage regimes, thus merely achieved goal-oriented optimisation with some negative constraints on specific nutrient (e.g. bioactive compounds, aroma volatiles).
- A critical threshold of 'CA' storage period, gas combinations and temperature that would create a good balance of nutritional profile including bioactive compounds, aroma volatiles and other related nutrients in addition to prolonged shelf life merits further investigations to meet a broad range of customer needs.
- The success of 'CA' technology depends on the understanding of the postharvest storage behaviour of pomegranate to determine a critical threshold beyond which physiological stress may not exceed irreparable level, which in turn may affect the fruit when taken out of 'CA' storage conditions.
- Generally, the establishment of critical and optimal O_2 and CO_2 gas combinations and temperature has potential to extend the shelf life of pomegranate. However, further work on optimal duration of exposure of pomegranate to 'CA' is fundamentally important for optimising specific nutrients such as bioactive compound and aroma volatile compounds.

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SUPPLEMENTARY TABLES

Table 3.2. Coefficient of variation (%)of treatments on texture profile before and after storage

Cultivar	Treatment	Whole fruit	Aril
‘‘Bhagwa’’	Day 0	0.5	2.1
	RA	0.1	4.0
	CA1	1.5	3.4
	CA2	0.3	5.2
‘Wonderful’	Day 0	0.8	2.7
	RA5	0.1	1.7
	CA1	0.3	1.6
	CA2	1.0	2.5

Coefficient of variation at $p<0.05$, RA=Room air at 5 ^{o}C

Table 3.3. Coefficient of variation (%) of changes in gas composition inside Xtend® film packaging during storage

Treatment	Gases	‘Wonderful’	‘Bhagwa’
XT5	O_2	5.6	6.2
XT5	CO_2	4.0	5.8
XT20	O_2	12.3	15.2
XT20	CO_2	15.9	25.4

Table 3.4. Coefficient of variation (%) of physiological disorder (decay) within treatments and cultivars

Treatment	'Wonderful'	'Bhagwa'
RA5	8.4	5.7
RA20	4.7	7.3
XT5	39.3	10.6
XT20	1.3	2.0
CA1	9.3	9.4
CA2	90.0	21.6

RA=Room air, XT= Xtend@ film, CA1=3% O_2 + 6% CO_2, CA2=5% O_2 + 14% CO_2

Table 3.5. Coefficient of variation on weight loss (percentage) within treatments and cultivars

Treatment	'Wonderful'	'Bhagwa'
RA20	37.2	27.7
RA5	18.5	20.6
XT5	32.7	27.0
CA1	33.3	12.8
CA2	15.6	15.8

RA=Room air, XT= Xtend@ film, CA1=3% O_2 + 6% CO_2, CA2=5% O_2 + 14% CO_2

Table 3.6. Coefficient of variations of TSS (°Brix) within treatments and cultivars

Treatment	'Wonderful'	'Bhagwa'
RA20	1.6	5.2
RA5	1.8	4.2
XT5	1.6	2.6
CA1	2.1	2.4
CA2	2.2	1.9

RA=Room air, XT= Xtend@ film, CA1=3% O_2 + 6% CO_2, CA2=5% O_2 + 14% CO_2

Table 3.7. Coefficient of variations (%) TA within treatments and cultivars

Treatment	'Wonderful'	'Bhagwa'
RA20	9.7	12.8
RA5	16.1	4.7
XT5	9.8	21.3
CA1	17.5	16.6
CA2	25.7	18.5

RA=Room air, XT= Xtend@ film, CA1=3% O_2 + 6% CO_2, CA2=5% O_2 + 14% CO_2